DISSERTATION

HISTOPATHOLOGICAL ANALYSIS AND STUDY ON EXPRESSION OF HER 2 AND Ki-67 IN GASTRIC CARCINOMAS

SUBMITTED FOR M.D DEGREE EXAMINATION

(PATHOLOGY) - BRANCH III

APRIL 2015

THANJAVUR MEDICAL COLLEGE

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

CHENNAI - TAMILNADU

CERTIFICATE

This is to certify that this dissertation by the title **"Histopathological Analysis and Study On Expression of HER2 and Ki 67 in Gastric Carcinomas "** is the original and bonafide work done by **Dr. R. Divya**, submitted as partial fulfillment for the requirements of M.D Degree Examinations - Branch III Pathology to be held in April 2015.

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Date : :09:2014.

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This is to certify that this dissertation entitled "**HISTOPATHOLOGICAL ANALYSIS AND STUDY ON EXPRESSION OF HER-2 AND Ki-67 IN GASTRIC CARCINOMAS**" is the original and bonafide work done by **Dr.R.Divya** under my guidance and supervision at the Thanjavur Medical college & Hospital, during the tenure of her course in M.D.Pathology from May 2012 to April 2015 held under the regulation of the Tamilnadu Dr.M.G.R Medical university, Guindy, Chennai-600032.

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submitted by Dr. R. DIVYA of

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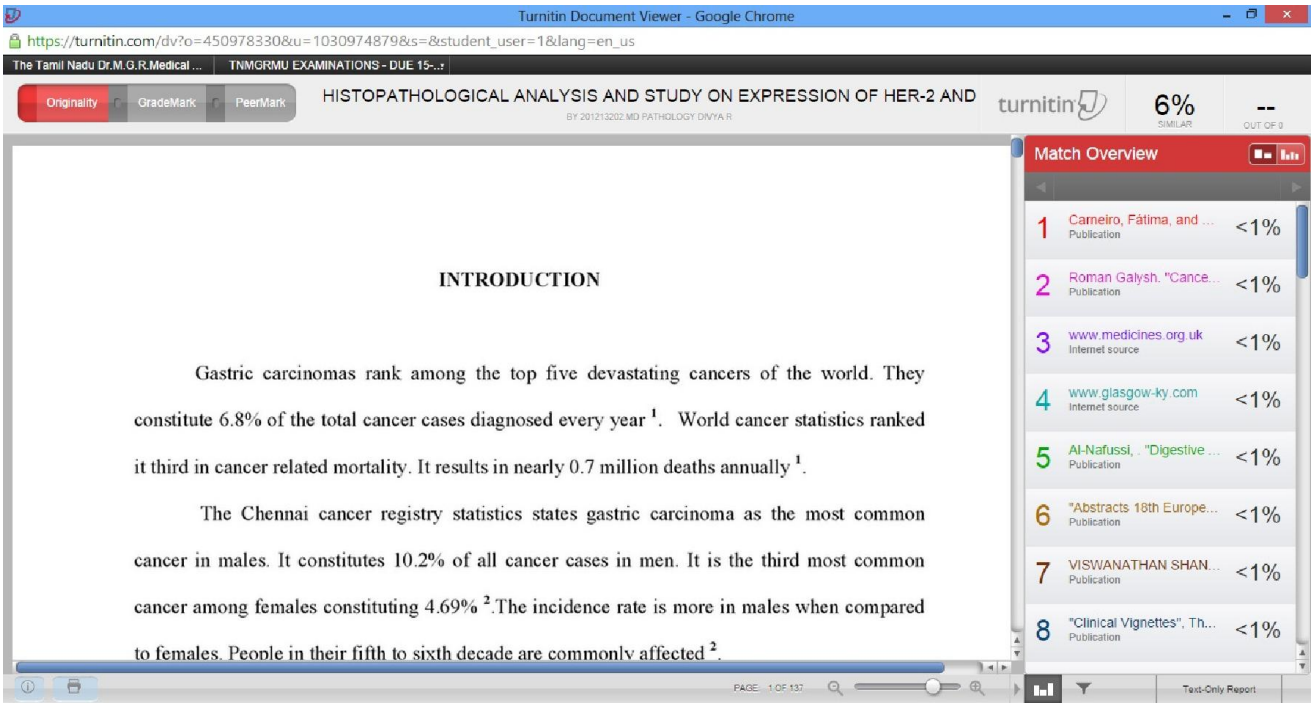
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INTRODUCTION
Gastric carcinomas rank among the top five devastating cancers of the world. They constitute 6.8% of the total cancer cases diagnosed every year [1]. World cancer statistics ranked it third in cancer related mortality. It results in nearly 0.7 million deaths annually [1].
The Chennai cancer registry statistics states gastric carcinoma as the most common cancer in males. It constitutes 10.2% of all cancer cases in men. It is the third most common cancer among females constituting 4.69% [2].The incidence rate is more in males when compared to females. People in their fifth to sixth decade are commonly affected [2].
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ABBREVIATIONS

AgNOR : Silver Nitrate- Nucleolar Organisation Region

Ano-1 : Anoctamin-1

APC : Adenomatous Polyposis Coli

AR : Amphiregulin

Bif : BAX interacting factor

BTC : Betacellulin

Cag A : Cytotoxin associated gene A

CEA : Carcinoembryonic Antigen

c-erbB 2 : erythroblastic leukemia viral oncogene homologue 2

CGH : Comparitive Genomic Hybridisation

CISH : Chromogenic Insitu Hybridisation

COX : Cyclooxygenase

DAB : Diamino benzidine

DLBCL : Diffuse Large B Cell Lymphoma

DNA : Deoxyribonucleic acid

DOG -1 : Discovered on GIST

EBER : Ebstein Barr Virus associated non polyadenylated RNA-1

EC Cell : Enterochromaffin cell

ECM : Extracellular matrix

EGFR : Epidermal growth Factor Receptor

ELISA : Enzyme Linked Immunosorbant Assay

EMA : Epithelial Membrane Antigen.

EPIYA : Glu- Pro-Ile-Tyr- Ala motif

EPR : Epiregullin

FGF : Fibroblast Growth Receptor

FHIT : Fragile Histidine Triad Protein

FISH : Flourescent In situ Hybridisation

Fn 14 : Fibroblast Growth factor Inducible Factor 14

GERD : Gastro esophageal reflux disease.

GFAP : Glial Fibrillary Acidic Protein

GIT :Gastro intestinal Tract

H.Pylori : Helicobactor pylori

HBEGF : Heparin Binding Epidermal Growth Factor

HCG : Human Chorionic Gonadotropin

HER 2 : Human Epidermal Growth Factor Receptor

HIA : High Iron Diamine

HLA : Human Leucocyte Antigen

hTERT : human Telomerase Reverse Transcriptase

IARC : International Agency for Research on Cancer

ICAT : Isotope coded affinity Tag

IHC : Immunohistochemistry

iTRAQ : isobaric tags for Relative and Absolute Quantitation

LINE(1) : Long Interspersed nucleotide element Type 1.

LOH : Loss Of Heterozygosity

MALDI : Matrix assisted laser desorption/ Ionisation mass spectrometry

MALT : Mucosa Associated Lymphoid Tissue

MAPK : Mitogen Activated Protein Kinase

MMP : Matrix Metalloproteinase

MSI : Microsatellite instability

mTOR : Mammalian Target of Rapamycin

MUC : Mucin

NEC : Neuro Endocrine Carcinoma

NET : Neuro Endocrine Tumour

NF-κB : Nuclear Factor kappa –B

NHL : Non Hodgkins Lymphoma

NRG : Neuregullin

OG junction : Oesophago gastric junction

ORAOV2 : Overexpressed in Oral Carcinoma

PAI : Plasminogen Activator Inhibitor

PAS : Periodic Acid Schiff

PCNA :Proliferating Cell Nuclear Antigen

PDGFR : Platelet derived Growth Factor

PTAH : Phosphotungstic Acid Hematoxylin

PTCH : Human Patched Gene 1

RT- PCR : Reverse Transcriptase Polymerase Chain Reaction

RUNX3 : Runt related transcription Factor 3

SELDI- TOF : Surface Enhanced Laser Desorption/ Ionisation Time of Flight Mass spectrometry

TFF 1 : Trefoil factor 1

TGF : Tumour Growth Factor

TIMP : Tissue Inhibitor if Metalloproteinase

TNF-α : Tumour necrosis factor alpha

TNM : Tumour, Node, Metastasis

t-PA : Tissue type Plasminogen activator

u- PA : Urokinase type Plasminogen activator

Vac A :Vacuolating cytotoxin A

VEGF : Vascular Endothelial Growth Factor

WHO : World Health Organisation

CONTENTS

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In the forthcoming decade gastric carcinoma would be the major killer disease. In India gastric cancer related mortality would raise upto 19.8% [3]. In spite of its magnitude gastric carcinomas have a wide geographic variability. Asians have increased incidence rate when compared to Caucasians and Africans.[4, 5]

Risk factors include H.pylori infections, diet, alcohol, tobacco and blood group 'A'. Medical conditions like menetrier's disease, gastric adenomas. Pernicious anaemia contribute to risk.[4, 6, 7, 8]

The most common site for the occurrence of gastric carcinoma is the distal end of the stomach. Recently increased incidence of carcinoma in the proximal end has been reported.[7,]

Gastric carcinomas lack specific symptoms in its early stage delaying timely diagnosis. These results in advanced stage of illness that has poor curability rates [4].The most commonly used diagnostic tool is the endoscopic guided biopsies .But they tend to miss out a considerable number of cases [4].

Majority of the gastric carcinomas are adenocarcinomas. Other variants are adenosquamous, neuroendocrine, mesenchymal tumours and lymphomas.[9]

Conventionally the prognosis of gastric carcinoma depends upon the TNM staging. This includes depth of invasion, nodal status and distant metastasis. Latest research works discovered special proteins expressed by the tumour cells. These proteins play a significant role in determining the prognosis. [10, 11, 12]

In this study carried out in Thanjavur medical college the histopathological analysis along with the immunohistochemical expression of two markers namely HER2 neu and Ki-67 were studied.

Ki-67 is a proliferation marker. It is related to the degree of differentiation, infiltration and lymph node metastasis. [12]

HER2 neu belongs to the family of epidermal growth factor receptors. It is overexpressed in a number of carcinomas. Its overexpression in gastric carcinomas is associated with more aggressive disease.[10, 11, 13]

AIMS & OBJECTIVES

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1. To study the incidence of gastric carcinomas reported in the department of pathology , Thanjavur medical college
2. To determine the age and sex wise distribution of gastric carcinomas of various histological types.
3. To analyse the anatomical distribution of gastric carcinomas.
4. To evaluate the expression of Ki-67 and HER 2 neu in gastric carcinomas.

MATERIALS AND METHODS

MATERIALS AND METHODS

A total of 260 specimens including endoscopic biopsies and gastrectomy specimens received in the department of Pathology, Thanjavur medical college during the period of July 2012 to June 2014 from the department of gastroenterology and Surgery were included in this study.

EXCLUSION CRITERIA

1. Chronic gastritis
2. Lesions in OG junction or in its proximal 5 cm
3. Nil tissue
4. Few dysplastic cells
5. Non neoplastic polyps
6. Ulcers with inflammation

The case details including the age and sex were recorded for all cases. Depending on the site of lesion, the stomach is dissected. It was opened either along the greater curvature or the lesser curvature. The specimen was pinned with its mucosal side up. It was fixed in 10% buffered formalin overnight. The specimen was then measured along its lesser and greater curvature. Any abnormal lesion such as ulcer, growth, flattening of mucosa was noted. Size, colour, shape, consistency, margins of ulcer, location, perforation if any, relation to the resected margins, relation to the visceral peritoneum was noted. The adjacent mucosa was examined for any gross abnormalities. The lesion was then cross sectioned to examine the depth of invasion [6,14].

SECTIONS FOR HISTOLOGY

1. Tumor proper – four sections through the wall including tumour border and adjacent mucosa
2. Non neoplastic mucosa in mid stomach – two sections
3. Proximal line of resection along the lesser curvature – two sections
4. Proximal line of resection along the greater curvature – two sections
5. Distal line of resection (along pylorus and duodenum if present) – two sections
6. Spleen if present
7. Pancreas if present
8. Lymph nodes – along the pylorus, lesser and greater curvature , perisplenic and omental nodes

The bits were subjected to routine tissue processing and paraffin embedding. Three to four micrometer sections were cut. The sections were stained with hematoxylin and eosin.(Appendix I). Entire gastric wall was visualized and the depth of invasion was determined. The sections were studied and histopathological parameters were evaluated according to the WHO classification criteria. Histochemistry was done with PAS stain to demonstrate the signet ring cells, in Carcinomas (Appendix II)

The immunohistochemical study was done using monoclonal primary antibody. They were directed against HER2 cytoplasmic membrane antigen and Ki-67 nuclear antigen. Sections of 4 micrometer thickness were used. The super sensitive TM polymer – HRP detection system was employed. It uses a non biotin polymeric technology. Here secondary antibody conjugated to poly-HRP reagent was bound to primary antibody. It can be visualized by DAB chromogen. (Appendix III)

HER 2 STAINING [15]

Grade	Surgical specimen Staining pattern	Biopsy specimen staining pattern	Her 2 Over expression assessment
0	No reactivity (or) Membranous reactivity in <10% tumour cells	No reactivity in any tumour cell	**Negative**
1 +	Faint or barely perceptible membranous reactivity in 10 % or more cells ; cells are reactive only in part of their membranes	Tumour cell cluster [a] with faint or barely perceptible membranous reactivity irrespective of % of tumour cells stained	**Negative**
2 +	Weak to moderate complete basolateral or lateral membranous reactivity in 10% or more of tumour cells	Tumour cell cluster with weak to moderate complete, basolateral or lateral membranous activity irrespective of % of tumour cells stained	**Equivocal**
3 +	Strong complete lateral or basolateral membranous reactivity in 10% or more of tumour cells	Tumour cell cluster with strong complete lateral or basolateral membranous reactivity irrespective of % of tumour cells stained	**Positive**

a - Tumour cell cluster is defined as cluster of five or more tumour cells

Ki-67 Immunostaining

The conventional four micrometer sections are cut from paraffin block. Immunohistochemical staining procedure is performed using the heat induced antigen retrival. Specific Murine Monoclonal Antibody – MIB-1 is used. Labeling Index is measured as percentage of MIB-1 positive cells in 1000 randomly selected tumour cells. The strong brown nuclear staining is considered positive. Weak nuclear or cytoplasmic staining is considered negative.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

"**Growth For The Sake Of Growth Is The Ideology Of The Cancer Cell**"

-Edward Abbey (1927 -1989)

American author and essayist

Popularly known as **"captain of men of death**" gastric carcinoma has its association with mankind from time immemorial.

Historical aspects [16]

- Cases resembling gastric cancer were first reported in the Ebers papyrus in 1600 BC
- Gastric cancer was mentioned in the reports of Hippocrates in the 2nd century AD in Rome.
- A condition resembling gastric cancer was described in Avicenna's medical encyclopedia in the 11th century.
- Benign and malignant gastric ulcer were described in 1835 by J.Cruveilhier.
- The death of Emperor Napoleon Bonaparte in 1821 was attributed to extensive scirrhous carcinoma of stomach . This was concluded based on the post mortem findings.
- The first gastric resection for cancer was performed by Jules Emilen Pean, a French surgeon in 1879.
- The first subtotal gastric resection with gastroduodenal anastamosis was performed in 1881 by Theodor Billroth.
- The first total gastrectomy was performed by Karl Schlatter in 1897.

Embryology [17]

During the fourth week of development stomach begins to form. It starts as a fusiform dilatation of foregut. It rotates 90º in its longitudinal axis. Hence making its left side lie anteriorly and right side lie posteriorly. There is rapid growth of posterior wall when compared to anterior wall. This results in the formation of lesser and greater curvature. Further rotation of the stomach along its anteroposterior axis occurs. Resulting in the caudal end moving towards the right and upwards. Cephalic portion to move towards the left and slightly downwards. This results in the final position of the stomach.

Anatomy [18, 19]

The stomach is situated in the upper abdomen. It extends from left upper quadrant, downwards forwards and to the right. It further extends towards the left hypochondriac, epigastric and umbilical areas.

Average capacity of the stomach at birth and at puberty 30 and 1000 ml respectively. Adult stomach has a capacity of 1500 ml.

The dome shaped fundus projects above and to left of cardiac orifice. It extends above the line drawn from incisura cardia to greater curvature.

The body of the stomach extends from the fundus to the incisura angularis. It is a notch in the lower end of lesser curvature. The lower boundary of the body of stomach is the line drawn from the incisura angularis to an indentation on the greater curvature. The position of the incisura angularis varies with gastric distension.

The pyloric antrum extends from lower boundary of body to sulcus intermedius. From here stomach narrows to form the pyloric canal. Pyloric canal measures around is 1-2 cm in length. The pyloric orifice is formed by marked thickening of circular muscular layer. Some fibres of longitudinal muscle coat also form pyloric antrum.

Lesser curvature gives attachment to lesser omentum. It contains right and left gastric vessels. Greater curvature gives attachment to the greater omentum. This contains gastroepiploic vessels and gastrosplenic ligaments.

Fundic mucosa extends into abdominal esophagus in a zigzag pattern forming Z line. The esophageal epithelium lies above this Z line. Z line is considered to be the gastroesophageal junction for histology and endoscopy.

The medial wall of stomach has the cardiac orifice. Its thickened mucosa forms a mucosal rosette that lines the orifice. Mucosa of fundus is thrown into gentle folds with no particular pattern. The body has more prominent mucosal folds. They appear as long broad ridges running from fundus to pyloric antrum. The magenstrasse are the least prominent smoother folds on the medial surface. They direct the liquid entering the stomach directly on the pyloric antrum. The antrum has a smooth mucosal surface. This culminates with puckering of the mucosa at the pyloric orifice.

Blood supply [19]

Celiac trunk, its branches hepatic and splenic arteries constitute the major blood supply.

The venous drainage of the stomach is divided into two portions. The proximal part drains into inferior esophageal plexus. The distal part drains into the portal vein.

Parasympathetic innervation is via the branches of vagus connecting Meissner and Auerbach plexus. The sympathetic innervations are via the celiac plexus.

The lymph nodes draining the stomach are numerous. They are right and left subpyloric, paracardial, splenopancreatic and gastroepiploic nodes.

HISTOLOGY [19, 20]

MUCOSA

Gastric mucosa is composed of a superficial layer and deep layer. The superficial layer is composed of foveolae or pits. They are formed due to invagination of surface epithelium. The deep layer is composed mainly of coiled glands. These glands empty in the base of foveolae. Glands in cardiac mucosa and proximal end of pyloric mucosa secrete mucus.

Surface Epithelium

The gastric mucosa is lined by mucus secreting tall columnar cells. They line the surface and the foveolae. The gastric glands are separated from the foveolae by the lamina propria. Foveolae in cardiac and pyloric end are wider imparting a villous architecture .The lining cells are tall columnar with a basally located nuclei. Nucleus is characterized by evenly distributed chromatin and inconspicuous nucleoli. The cytoplasm is superficial in location and filled with mucus.

Cardiac & Pyloric Mucosa

Half of the mucosal thickness is occupied by the foveolae. The glands are mucus secreting and loosely packed. Parietal cells are found in isolated small clusters at the pyloric region. Pyloric glands secrete only neutral mucin. Cardiac glands secrete both neutral mucin and sialomucin.

Cardiac mucosal abnormalities arise in setting of H.pylori infection or GERD. This results in inflammatory nuclear atypia, intestinal metaplasia and a hybrid mucosa. Hybrid mucosa is multilayered with surface columnar epithelium and basal squamous cell.

Fundic Mucosa

Fundic mucosa is also known as the Oxyntic mucosa. The foveola occupies less than ¼ th of the mucosal thickness. The glands are straight and tightly packed. They are divided into three portions

Base – Composed of zymogenic cells. They are cuboidal cells with pale blue gray cytoplasm. They have a basally situated nucleus and a small nucleolus.

Isthmus - composed of triangular parietal cells with their base lying on basement membrane. They have an eosinophilic cytoplasm and a centrally placed nucleus.

Neck – composed predominantly of a mixture of zymogenic and parietal cells. These cells are admixed with mucous neck cells.

Endocrine cells in stomach

Hormones from endocrine cells enter blood directly or exert a paracrine effect.

Antrum - 50 % - Gastrin producing **G** cells

30% - Enterochromaffin cells

15% - **D** cells

Fundus – Predominantly enterochromaffin like cells

X cells.

Enterochromaffin cells.

Lamina propria

It provides structural support. Composed of framework of reticulin, collagen and elastic fibres. Also contain cells like fibroblasts, histiocytes, plasma cells and lymphocytes. It also contains capillaries, arterioles and non myelinated nerve fibres. Occasional lymphocytes and plasma cells of B cell lineage may be found. They secrete IgA antibodies.

Muscularis Mucosa

They are composed of inner circular and outer longitudinal smooth muscle bundles. These muscle fibres are admixed with some elastin fibres. They are most obvious in antrum.

Submucosa

Core of gastric rugae is composed of submucosa, loose connective tissue, elastic fibres. They also contain autonomic nerve plexus of meissner, veins, arteries and lymphatics.

Muscularis Propria

Composed of outer longitudinal, inner circular and innermost oblique smooth muscle fibres. The fibres in outer longitudinal layer are continuous with the longitudinal layer of esophagus. The pyloric sphincter is formed by the aggregation of fibres of inner circular layer. The oblique layer is incomplete and is appreciable only in the cardia. The muscularis propria is thicker in the pyloric antrum.

Subserosa

Subserosal region has small blood vessels, fat cells and inconspicuous mesothelial cells.

Serosa

The gastric serosa is continuous with the greater and lesser omentum.

CELLS OF GASTRIC EPITHELIUM

Chief cells

Cuboidal cell with round euchromatic nucleus, basophilic cytoplasm and secretory zymogen granules. The chief cells secrete digestive enzymes pepsin and lipase. They are situated in the basal portion of the glands.

Parietal cells / Oxyntic cells

Large eosinophilic cell with a centrally placed nucleus. They secrete gastric acid and intrinsic factor and have a unique ultrastructural feature. Their luminal side is invaginated by blind ended channels with numerous microvilli. These microvillus contain H^+/K^+ atpase anti-porter channels. They secrete hydrogen ions into the lumen. The mitochondria rich cytoplasm contains tubule vesicular system.

Mucus Neck Cells

They are numerous at the neck and at the basal regions. It has mucin containing apical secretory vesicles.

Stem cells

They are located in the neck of gastric glands. Some are also located in the base of gastric pits. Not normally identified after damage to the gastric mucosa.

Neuroendocrine cells

They occur in the body and fundus among chief cells. These cells contain a basally situated irregular nuclei and granular cytoplasm. Cytoplasmic secretory granules are essential for gut motility and glandular secretion.

Functions Of Stomach

Fundus - Act as a pacemaker for co-ordinated contraction

Cardia - Helps in propulsion

Antrum - Crushing and grinding function

SPECIAL STAINS[19]

Giemsa stain highlights mast cells, H.Pylori, Giardia, Cryptosporidium. H.pylori is also demonstrated using **warthin starry stain** or the Steiner modified silver technique. Fungus is demonstrated using **PAS** after diastase application or methanamine silver technique.

The neutral mucins are better demonstrated with PAS with diastase. Acid sialomucin are demonstrated with Meyers mucicarmine and **alcian blue** at ph 2.5.

The sulphated mucin can be demonstrated by high iron diamine (HID) stain or alcian blue at pH of 1.

Endocrine cells

Argentaffin – Masson Fontana

Argyrophilic - 1. Grimelius

2. Churukian.

3. Schenk.

ENDOSCOPIC EXAMINATION and BIOPSY [7,19,20]

Endoscopy is often the first line investigations in gastritis and gastric ulcer.It helps in diagnosis and in also management of ulcer.In addition to diagnosis it helps in monitoring cases following therapy and complications.

Endoscopic surveillance is used for screening in high incidence areas of gastric carcinoma. Japanese employ a technique of applying dyes like Toluidine blue or congored. It distinguishes between normal and dysplastic gastric mucosa.

Endoscopy helps in sampling the lesion. It provides information regarding colour, texture and degree of vascularity of mucosa.

EPIDEMIOLOGY [5, 21,22,23]

Gastric carcinoma is the fourth most common cancer in the world. It is the second leading cause of cancer mortality. Inspite of its magnitude its incidence varies in different parts of world. Its predominant in developing countries when compared to developed western countries.

The occurrence of gastric carcinoma peaks in the sixth to seventh decade. It is uncommon in the younger individuals.

Recently there is an increasing incidence of gastric carcinoma in the proximal stomach. They are distinct from conventional tumours with more incidence in younger age. It is associated with conditions like hiatal hernia and GERD. They are less commonly influenced by ethnicity, diet or family history.

In spite of the number of diagnostic modalities gastric cancer mortality remains high. Most of the gastric cancers remain undetected in the early stage. Paucity of clinical symptoms delays diagnosis resulting in significant morbidity and mortality.

Risk Factors [7,8]

According to the International Agency for Research on Cancer (IARC) the risk factor for gastric carcinoma has been broadly categorised into

Factors with convincing evidence for increased risk

H.pylori infection, tobacco chewing, X rays, Gamma rays and working in rubber factories

Factor with probable evidence

Nitrate or nitrites, pickled vegetables, salted fish,salty foods, Asbestos, Inorganic lead compounds, Epstein barr virus.

Helicobacter pylori infection

Considerable cases of gastric adenocarcinoma is associated with multifocal atrophic gastritis. This is mainly due to H.pylori infection. H.pylori is not directly carcinogenic it aids tumorigenesis. It acts by

1) Intestinal metaplasia,

2) Cag A or Vac A positive strains of H.Pylori causes uncontrolled cell proliferation or apoptosis.

3) Hypochlorhydria resulting from atrophic gastritis results in overgrowth of anaerobic bacteria.This converts reactive nitrate intermediates into carcinogenic substances.

4) The reactive oxygen species are released during inflammation.They damage lipids, DNA ,proteins and oxidises antioxidants like vitamin C.

Individuals with HLA-DQA1*0102 genotype are resistant to H.pylori induced intestinal metaplasia and are at decreased risk for gastric adenocarcinoma

Dietary factors

Soyabean products consumed in Asia combined with sodium nitrite is mutagenic. Salty food damages gastric mucosa and enhances cell proliferation. Consumption of processed meat enhances the risk of gastric carcinoma. Consumption of fresh fruits and vegetables decreases the risk of carcinoma. Tobacco is a proven risk factor. Carcinogenic potential of alcohol in causing gastric carcinoma is not clearly evident.

Influence of dietary factors in gastric carcinoma in India[35,36,37,38]

Diverse ethinicity and cultural variation in India reflects in local cusine. This plays a major role in determing the risk of gastric carcinoma. Incidence of gatric carcinoma is high in the southern parts of india. South Indians are more affected compared to the population in the north.

Low intake of fresh fruits and high prevalance of smoking and alcoholism are important determinants of stomach cancer in the rural population of India.According to Imran et. al.[35] High cooking temperature of Indian dishes destroys vitamin C. This decreases protective anti oxidant effect. Cancer incidence is high in north eastern states of India like Sikkim, Arunachal pradesh and Nagaland. Increased consumption of smoked meat increses the risk. Tuibur, a local beverage which is a filterate of tobacco in water increases the risk of gastric carcinoma.

Increased intake of brassica vegetables, chillis and salted tea in kashmiris increases the risk. This increases content of dietary amines and carcinogenic nitrocompounds. Drinking tea is said to have a protective effect on gatric mucosa. Increased consumption of coffee increased the chance of acquiring gastric carcinoma by 20%. But this data is not statistically proven.

Intestinal type of adenocarcinoma is commonly associated with smoking. Consumption of tobacco and smoking comes in various forms. Bidi is a local cigar produced from sun cured leaves of tobacco.It is rolled in sheets of dried temburni leaves (Diospyros melanoxylon). It has a nicotine content of roughly 0.2 – 0.3 gm^3. Chutta or cheeroot is a smaller form of cigar .It is made by rolling tobacco flakes in sun dried tobacco leaf. Snuff is an inhalent form of tobacco.This comes in the form of a fine dried powder. Quid is the chewable product made from vine piper betal. It is chewed with bits of areca nut and small amount of lime. Hookah smoking is a traditional form of smoking practised in kashmir. This is a mixture of tobacco

leaves, honey and other local fruits . Habitual hookah smoking is associated with increased risk of developing cancer.

Increased risk of gastric carcinoma is seen among smokers of bidi. The content of tobacco is less in bidi when compared to cigars. Tar, benzene compounds and hydrocarbons are carcinogenic. Decresed Porosity of the Leaf, low combustibility and high Concentration Of Volatile Phenols increase the risk.

A variety of liquors are cosumed in India. Toddy is the fermented palm sap produced locally. Arrack is a locally brewed liquor with 40% ethanol content. Other drinks are wine, whiskey, beer, brandy,gin and rum. Increased risk of gastric carcinoma is associated with increased arrack consumption. Risk of gastric carcinoma is increased with consumption of Vodka and red wine.

Nagaraj et al states that in a study carried out in Tata memorial hospital it has been conclude that consumption of wine increases the risk of development of carcinoma when compared to individuals who consume beer, brandy, rum and tequila.

Increased intake of fruits and vegetables has negative corelation to occurance of gastric carcinoma. The high levels of antioxidants protects gastric mucosa by inhibiting the nitration process. Vegetables and meat that has been salted ,pickled or smoked are carcinogenic. They act as abarasives in gastric mucosa elevating the levels of nitrosocompounds. Foods like allium, garlic, onion, green tea protects gastric mucosa. Individuals with pernicious anaemia has increased tendency to develop papillary lesions of stomach.Nagaraj et al states that increased consumption of dried fish increases the risk of gatric carcinoma.

Occupational risk

In a study carried out in Australia occupational risks were analysed. Subset of population who practice agriculture have decreased risk for developing carcinoma. Individuals

occupied in news printing have increased due to lead exposure. Increased risk is present in grinder operators exposed to mineral oil & ethanolamine. Individuals involved in electrical and electronics industry, pavers and excavators have increased risk.

GENETIC FACTORS [23,24,6,26]

Genetics of gastric carcinoma[6,23,24,26]

12% of gases of gastric carcinoma show familial inheritance. The most popular example is Emperor Napolean Bonaparte and his family members .They were believed to have succumbed to schirrous variant of gastric carcinoma.

Swedish Family cancer database documents the standerdised incidence ratio in a child whose parent was affected by gastric carcinoma as 1.59. In case of a sibiling it is 5.75%. This implies that risk in sibilings is higher when compared to offsprings.

Inherited predisposition syndromes

1-3% of all cases of gastric carcinoma come under this category.

Heriditary diffuse gastric cancer

Cases of gastric carcinoma with an autosomal pattern of inheritance and high penetrance was observed in tha Maori families Linkage Analysis Was Performed. It revealed mutations in E-Cadherin/CDH locus on long arm of chromosome 16 at position 22. Till date multiple E-Cadherin mutations has been noted. These mutations are found distributed among nearly 16 exons. 70% of them being truncated mutations and 30% are missense mutations. Age of onset ranges from 15 to 70 yrs with an average age of around 40 years. Females with this mutation has an 60% increased risk of acquiring lobular carcinoma of breast. They are prone to develop the tumour before 80 years of age. Incomplete penetrance in mutation of this kind does not seem to

produce carcinoma. Prophylactic gastrectomy and rigorous surveillance of breast cancer is recommended. Individuals harbouring E Cadherin mutations should be screened from 20 years of age.

1. Lynch syndrome

This occurs due to a mutation in germline mismatch repair gene. Usually produces intestinal typeof adenocarcinoma with no evidence of H.pylori infection. The mean age of occurance is 56 years

2. Li Fraumeni syndrome

It is charecterised by occurance of multiple tumours. They include sarcomas, carcinoma breast, CNS tumours, leukemias, gastric carcinomas and adrenocaortical tumours. This is attributed to germline mutation in $P_{53.}$

3.Peutz Jeghers Syndrome

It is caused by germline mutation in STK11.It is an autosomal dominant disorder. Charecterised by pigmentation of digits,lips,buccal mucosa, hamartomatous polyps and carcinomas of gastrointestinal tract.

Gastric carcinomas are also part of the Juvenile polyposis syndrome in individuals who harbour mutations in SMAD 4 or BMPR11A.

Genetic alterations in gastric cancer

Gastric mucosa is composed of numerous multipotent stem cells that forming clonal units. The crypts showing intestinal metaplasia showed clonality. They are composed of multiple pluripotent stem cells that spread by crypt fission. Frameshift mutation in TGFβ II, IGF II R, BAX, MSH6, E_2F4, MSH 3 with microsatellite instability is seen in subset of individuals with

intestinal type carcinoma. Mutational amplification of HER2 gene, TP53 mutation results in overexpression of hTERT. About 60% of intestinal type carcinomas exhibit hypermethylation in RUNX5.

Chromosomal Instability

Nearly 72% of differentiated tumours and 43% of undifferentiated tumours showed aneuploidy. The other genetic aberrations are rearrangements in chromosome3, deletions distal to 6q21, trisomy8, monosomy 13, aberrations involving 11q13-p15 and translocations.

CGH studies demonstrated decrease in number of DNA copies in chromosomal arms 4q,5q,9p,17p, 18q. Increase in DNA copy number in chromosomes 8q,17qand 20q. The loss of genetic locus of 3p,4p,4q,5p,8q,13p,17p,and 18q has been demonstrated by Comprehensive Loss Of Heterozygosity (LOH) analysis.

Microsatellite instability

14-44% of sporadic gastric carcinomas are associated with microsatellite instability. MSI-H have loss of expression of MLH/MSH2. The silencing of hypermethylation is said to play a pivotal role. It is demonstrated by incresed methylation in the promoter region of MLH1 or MSH-I. The unique features of these tumours are all are distally located.They are predominantly of intestinal type and is of good prognosis.

The defective mismatch repair in MSI-H tumours targeted many important proteins namely TGFβ type II receptor (TGFβR2), ACVR2. Other genes affected are IGFRII, hMSH3, hMSH6 and E2F-4 involved in regulating progression of cell cycle and signalling apoptosis.

AQUIRED SOMATIC GENETIC/ MOLECULAR ALTERATIONS

TFF-1 Loss :

50% of gastric carcinomas demonstrate mutations in TFF1(pS2). Trefoil peptide is a molecule with three loops produced by the mucus secreting cells. This is present in chromosome 21q22. This is associated with gastrokine (GKN)2. This ia a tumour suppressor gene specifically associated with stomach.

E-Cadherin Alteration :

E-Cadherin is a transmembrane calcium ion dependent adhesion molecule. It is associated with sporadic diffuse gastric cancer. It is involved in homotypic interaction of epithelial cells. The invasive properties of gastric carcinoma is associated with loss of E-Cadherin. Decreased expression of this protein in the gastric mucosa when compared to adjacent normal gastric mucosa. Methylation of the promoter region of E-Cadherin was found in many cases.

Kinases /Phosphatases:

P13Kis a lipid phosphatidoyl inositol kinase. It is involved in cellular proliferation, adhesion, survival and mortality.

Methylation silencing alteration:

Mutations resulting in methylation of the CpG island in the promotor region of p16. The tumour suppressor gene RUNX3 inhibits the epithelial cell growth.

Apoptosis signalling alterations:

BAX gene promoting apoptosis is mutated in 33% of gastric carcinomas. It is often associated with loss of expression of Bif-1(BAX-interating factor) that disrupts the apoptotic pathway. Another promoter that upregulates apoptosis is Bcl 2 homologue BAK. Defective expression of cell surface death receptors DR4 and DR5 has been observed.

P53 mutations by allelic loss has been observed in gastric carcinomas. They involve mainly the base transitional mutations involving CpG nucleotides.

HER2/neu which was found to be overexpressed in many gastric carcinomas. It is said to have interactions with CD44. CXCR4 is upregulated via epigenetic silencing of micro rna 139. VEGF polymorphisms in gastric carcinomas is associated with poor prognosis. Other genes implicated are FHIT, chromatic remodelling gene ARIDIA, members of Wnt signalling pathway, APC and β- catenin, Hedgehog Target Genes, Human Patched Gene 1 (PTCH1).

Proteomics in gastric carcinoma

With invention of new techniques like Protein Chip Array, 2Dimensional electrophoresis, Liquid chromatography, iTRAQ (isobaric Tags for Relative and Absolute Quantitation), ICAT (Isotope Coded Affinity Tag) proteomic analysis of various abnormal proteins expressed in gastric cancers could be done. These involve proteins that act as check points in mitosis such as MAD1L1,EBI,HSP27,CYR61 and CLPP.

SELDI-TOF-MS is Surface Enhanced Laser Desorption /Ionisation Time Of Flight Mass Spectrometry.It is a technique that demonstrated upregulation of proteins like pepsinogen C and pepsin. It downregulates α- defensins in gastric carcinoma. Fn14 (Fibroblast growth factor inducible factor 14) of the TNF family is over expressed in gastric carcinoma.

MALDI- Matrix assisted laser desorption/ ionisation mass spectrometry done mainly on endoscopic biopsies. It uses a unique protein profile that serves as a molecular signal. It is composed of 73 signals. It serves to distinguish gastric carcinoma from normal gastric mucosa.

Many proteins serve to distinguish normal gastric tissue from carcinoma. They either getting upregulated as in case of Notch 4, Akt and β catenin. They are downregulated as in Cyclin E, p27, E-Cadherin,HIF-3a, NF-KB. Altered expression of HIF-3a and NF-KB is associated with increased invasiveness.

Biomarkers :[26]

Serum markers like CEA and CA 10-9 has got little specificity and sensitivity. New biomarkers like Urokinase Type Plasminogen Activator (U-PA), VEGF, MET, MYC,tie-1 protein,tyrosine kinases, CD44r6, PDGF-A,TGF-β and cyclin D were studied. Their increased levels are found to be associated with incresed invasiveness. Decresed levels of proteins like Kip 1, CIP1, PAI-I and tPA are associated with increased invasive properties.

Chemosensitisation markers:[26]

1. Overexpression of p53 in advanced gastric carcinomas predicted lower responsive rates to chemotherapy.
2. Increased expression of thymidine synthase or phosphorylase predicts outcome of flurouracil therapy.
3. Tumours with positive staging for BAX and Bcl2 has got poor prognosis. They show resistance to chemotherapy drugs.

Serum markers:[26]

Increased serum levels of TIMP-1, hepatocyte growth factor, soluble receptors for interleukin 2, interleukin 10, soluble fragments of E Cadherin in gastric carcinoma patients has been associated with decreased survival rates.These tumours have greater degree of tumour invasion.

PRECURSORS OF GASTRIC CARCINOMAS [7]

Gastric carcinoma precursors are broadly classified into precancerous conditions and precancerous lesions.

PRECANCEROUS CONDITIONS

These are clinical conditions associated with incresed risk of gastric carcinoma. Patients with these conditions do not develop gastric carcinoma. They are

1.Epithelial polyps

2. Intestinal metaplasia

3.Chronic gastric ulcer

4. Gastric remnants

5. Hyperplastic gastropathy.

PRECANCEROUS LESIONS

These are the pathological conditions which eventually evolve into gastric carcinomas.

H.pylori associated chronic gastritis

H.pylori has been declared by the WHO as a class I carcinogen.It is the only bacterium to assume this status. It causes chronic infection in more than 50% of the population. Its association of is proven in more than 80% of the cases of gastric carcinoma. Statistics state that only 1-3% of individuals with H.pylori infection develop gastric cancer. Host factors coupled with H.pylori infection is necessary for the development of carcinoma.

H.pylori infection is often acquired during infancy and persists for life. It colonizes the gastric mucosa eliciting an immunological response in the host.It causes multifocal atrophic gastritis. The virulence of this gram negative bacteria vary. The strain containing the Cag A gene (Cytotoxin associated gene) codes for an oncoprotein. This is injected by type IV secretion system into the gastric epithelial cells. Once inside the cell it becomes phosphorylated in motifs containing EPIYA sequence. This starts a chain of events leading to carcinogenesis. This Cag A gene disrupts the intercellular junction. It causes loss of polarity, increases proliferation, reduces apoptosis and leads to carcinogenesis.

The organism has another gene the Vac A (Virulence associated gene) . This causes apoptosis by introducing cytoplasmic vacoules and pores in cell membrane.

The process of infection generates reactive oxygen species that incites DNA mutation. It causes hypermethylation of DNA in the CpG islands. This results in gene silencing associated with tumour suppresion.

The bacterial membrane has an adhesion protein Bab A (Blood group antigen binding Adherin). This adheres to the lewis antigen present in the epithelial cell membranes. This confers greater risk of cancer.

Autoimmune Gastritis

It accounts for less than 5% of chronic gastritis. This condition results from the immune mediated destruction of parietal cells. It is restricted to the body and fundus. It causes neuroendocrine cell hyperplasia resulting in Type I Neuroendocrine tumours. Destruction of parietal cells results in lack of intrinsic factor.This leads to Vitamin B12 deficiency, causing perinicious anaemia. These individuals have 3 fold increased risk of developing intestinal adenocarcinoma.

Atrophic Gastritis

The loss of normal glandular epithelium occurs in two ways. In one gland is destroyed and replaced with lamina propria fibrosis. In the other, glandular loss results from replacement of the normal glands by metaplastic epithelium.

In H.pylori gastritis this process occurs in the gastric antrum.It occurs in mucosa of fundus and body in autoimmune gastritis. This may progress to advanced atrophic pangastritis.

METAPLASIA

It is the reversible condition. Here one mature cell type is replaced by another mature cell. This occurs inorder to prepare the mucosa to withstand adverse conditions. Various types of metaplasia occur in stomach.

Spasmolytic Polypeptide expressing Metaplasia

This metaplastic process resembles deep antral glands but lacks gastrin producing cells. Also called antralised oxyntic mucosa, mucous metaplasia or pseudopyloric metaplasia.These metaplastic cells express TFF 2 (Trefoil factor 2).

TFF are proteins that aids in protection and repair of gastrointestinal tract. TFF1 and TFF2 is expressed in stomach. TFF3 also called intestinal trefoil factor. Expressed by Goblet cells of intestine in normal condition .

Recent studies have proven a strong association of SPEM in 90% of gastric cancer.It is believed to be the first change that preceeds intestinal metaplasia. Role of SPEM as precursor of carcinoma or an associated entity in the carcinogenic process is yet to be proven.

Intestinal Metaplasia

It is of three types

Type I

It is also called complete or small intestinal type. Consist of absorptive cells, Paneth cells and Goblet cells secreting sialomucin. There is increased expression of intestinal mucin MUC 2. There is decreased expression of gastric mucin MUC 1, MUC5AC and MUC 6. It is the most predominant subtype and seen mostly in benign conditions.

Type II

It is also called incomplete, immature or the colonic type. Charcterised by lack of absorptive cells. There is presence of varying stages of differentiation of columnar or intermediate cells. They secrete neutral and acid sialomucin and goblet cells. The predominant mucin type is Sialomucin.

Type III

The predominant cell type is Sulphomucin secreting intermediate cells.Percentage of association of type III intestinal metaplasia with gastric carcinoma is often less than 10%.

Gastric polyps [26]

The epithelial polyps of the stomach are of many types. They are hyperplastic, inflammatory, heterotopic and hamartamatous polyps. Majority of the gastric polyps are fundic gland polyps. They can occur sporadically or as part of familial adenomatous polyposis syndrome. A recently diagnosed entity is an Autosomal dominant condition termed Gastric adenocarcinoma and proximal polyposis syndrome.It is characterised by development of numerous fundic gland polyps. There is associated dysplastic changes in the gastric epithelium.

Hyperplastic polyps are found in the setting of H.pylori infection.They are associated with dysplasia in 1-3%.

1-4 % of the gastric polyps are adenomas. They are classified based on architechture as tubular, villous or tubulovillous. Based on epithelial phenotype they are subclassified into gastric and intestinal type adenoma.

Intestinal type adenoma

They are common in males, often occuring in antrum. Composed of cells with elongated hyperchromatic nuclei, goblet cells and paneth cells. Associated with chronic gatritis and intestinal metaplasia.They have a high risk for malignant transformation.

Gastric adenomas

Foveolar type

Occurs in association with Familial Adenomatous Polyposis. Present predominantly in body. Composed of cells with oval to round nucleus, pale cytoplasm. They contain apical mucin.

Pyloric type

More common in females. Associated with autoimmune gastritis and intestinal metaplasia. Has a high risk for malignant transformation. Composed of cells with round atypical nuclei with ground glass cytoplasm.

Oxyntic type adenoma

Negligible risk for malignant transformation. Composed of chief cells and mucous neck cells.

Chronic gastric ulcer [7]

Cancer involving the margin but not the base of a chronic ulcer. Ulcer-cancer was a term coined by Hauser in 1926.. This term is not commonly used nowadays. A chronically ulcerated epithelium is more susceptible to carcinogenic stimulus. This is postulated to be the reason for this phenomenon.

Gastric remnants [7]

The increased incidence of carcinoma has been reported in a subset of individuals. These group of individuals underwent Billroth II gastrectomy for duodenal ulcer.The incidence was

influenced by the operation – carcinoma interval. Usually develops nearly 10 years following the surgical procedure. The stoma is the most common site. Both type of carcinomas -intestinal and diffuse has been reported. The probable mechanism of carcinogenesis is the loss of sphincteric action. The gastro enterostomy stomal site is thus subjected to a constant bile exposure. This causes mucosal abnormalities eventually turning to gastric carcinoma.

Hyperplastic gastropathy [7]

The thickening of the gastric mucosa in seen in two conditions

Zollinger Ellison syndrome

There is glandular hyperplasia with hyperacidity . No risk of malignancy.

Menetriers disease

There is mucus cell hyperplasia with protein loss. Probable risk of malignancy.

Chronic hypertrophic glandular gastritis of schindler

Increase in the normal looking glandular elements.

PRECANCEROUS LESIONS OF STOMACH [7]

These conditions which precedes the development of carcinoma. Eg.,Dysplasia.

Dysplasia

Abnormal state of tissue charecterised by pronounced cellular and structural alterations. They have increased propensity for malignant transformation.

Various studies have classified dysplasia they are,

1) **Cuello classification**

   Hyperplastic type

   Adenomatous type

2) **Jass classsification**

   Type I or Adenomatous lesion

Type II or Non adenomatous lesion

3) **Vienna classification** [90]

Category 1 – Negative for neoplasia / dysplasia

Category 2 – Indefinite for neoplasia / dysplasia

Category 3 – Non invasive low grade neoplasia

Category 4 – Non invasive high grade neoplasia

Category 5 – Invasive neoplasia

Intramucosal carcinoma

Submucosal carcinoma

4) **WHO Classification**

Low grade dysplasia

High grade dysplasia

Low grade dysplasia

- Confined to superficial part of mucosa.
- Composed of simple dysplastic tubules with little branching
- .They lie underneath the normal foveolar epithelium.
- The cells have a basal nucleus with a small indistinct nucleoli
- Sparse mitosis

High grade dysplasia

- Architectural changes like elongation, budding often producing cribriform pattern.
- The cells have an enlarged Vesicular nucleus with irregularly clumped chromatin and irregular nucleoli. The nuclear cytoplasmic ratio is increased with loss of nuclear polarity.

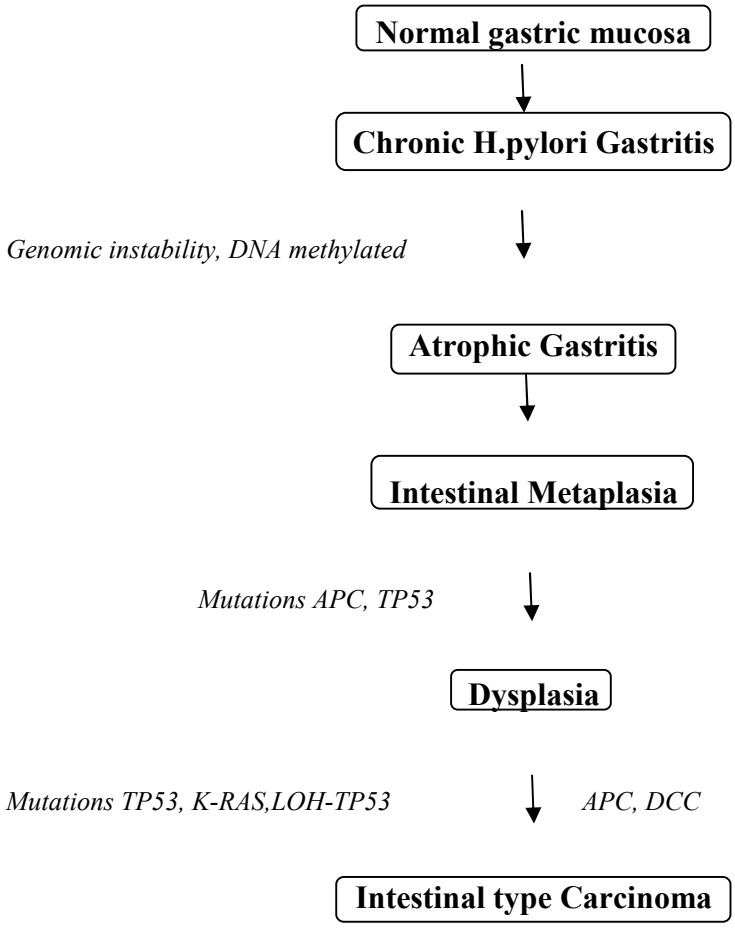
Intramucosal Adenocarcinoma

The neoplastic cells invade into the lamina propria or the muscularis mucosa. They don't invade the submucosa.

Criteria for invasion

Presence of single cells or clusters, budding of tumour cells into the lamina propria. There should be architectural distortion with fusion of glands. This condition should be differentiated from high grade dysplasia.

The **Correa cascade**[26] is a chain of events believed to occur following a H.pylori infection. Over a period of years it culminates in adenocarcinoma.



CLASSIFICATION OF GASTRIC ADENOCARCINOMA

Early gastric cancer[7]

It is the primary carcinoma confined to the mucosa and submucosa regardless of nodal metastasis. The term early implies the feasibility of complete surgical resection. It is not related to the time period of evolution. 86 % of the lesions are larger than 2 cm. Endoscopic screening helped identifying small cancers (<10 mm in size) and minute cancers (<5 mm) in size.

Macroscopic classification

Type I – **Protruding type** – An irregular nodular lesion with clefts between papillary projection.

Type II

IIa – **Superficial elevated type** – Mucosal elevation twice its normal size to 5mm.

IIb – **Superficial flat type –**Lesion is at the same level with surrounding mucosa.

IIc –Superficial depressed Lesion presents as a shallow depression. It is the most common type.It is further classified by Nagayo et al into

IIC - <3cm, well demarcated lesion.

IIC ' - >3cm, poorly demarcated, also called superficial spreading type.

Type III – Elevated ulcer with a strip of carcinomatous epithelium forming the border. Extremely rare.

Majority of the early gastric cancers are of tubular or signet ring type. Presence of an ulcer in a lesion of <3 cm aids in its conversion into a submucosal malignancy. In contrast to

lesions > 3cm where submucosal invasion occurred in the absence of ulceration. The presence of ulceration accelerates nodal metastasis.

Lymph node invasion

The extent of nodal metastasis depends on the size of primary tumour. For tumour <1cm diameter the risk is 4% and for tumour >4cm the risk increases to 18%. Metastasis was confined to primary regional nodes in lesions confined to mucosa. Lesions with extension into the submucosa involves the secondary and tertiary nodes.

CLASSIFICATION SYSTEMS USED IN GASTRIC CANCER [7,25,26]

I. Borrman's system

It is based on gross appearance of the tumour.

- **Superficial carcinoma** – Type 0 / early carcinoma
- **Polypoidal** – Type 1 / Nodular broad based growth without ulcer
- **Fungating** – Type 2 / Nodular lesion with an ulcer whose base lies above the stomach level.
- **Ulcerated** – Type 3 / Excavated lesion penetrating stomach wall. They have tumour cells in their base and margin.
- **Diffusely infiltrative** – Type 4 / Linitis plastica – Thickening of the stomach wall without obvious growth or ulceration. Also called scirrhous carcinoma

II. Lauren's classification

It is based on microscopic appearance

- **Intestinal type** – Resembles a colonic carcinoma. Common in older male patients and have a high rate of survival.
- **Diffuse type** – Tumour cell infiltrates as individual cells in a diffuse manner. Common in young females.
- **Intermediate type**

III. Mings classification

- **Expanding** – Grows as a well defined nodule or mass. It compress the near by tissues.Associated with high lymphocytic infiltration.
- **Infiltrative** – Grows as diffuse infiltration of individual tumour cells. Associated with intense desmoplasia.

IV. Nakamura classsification – on the basis of gland formation.

- Differentiated
- Undifferentiated

V. Mulligan classification[89]

- Intestinal type
- Mucous cell
- Pyloro cardiac

VI. Goseki's classsification [25]

I- well differentiated tubules, intracellular mucin poor

II - well differentiated tubules, intracellular mucin rich

III- Poorly differentiated tubules, intracellular mucin poor

IV- Poorly differentiated tubules, intracellular mucin rich

VII. Carneiro classification – histological classification

- Glandular
- Solid – has good prognosis
- Isolated
- Mixed

VIII. Adachi classification

Better prognosis - Tubular

Solid

Mucinous tumours with well differentiation

Poor prognosis - Signet ring type

Scirrhous

Mucinous tumours with poor differentiation

IX. WHO CLASSIFICATION OF GASTRIC CARCINOMA[4]

EPITHELIAL TUMOURS

PREMALIGNANT LESIONS

- Intraepithelial neoplasia (dysplasia),low grade
- Intraepithelial neoplasia (dysplasia),high grade

CARCINOMA

- ✓ Adenocarcinoma
  - Papillary adenocarcinoma
  - Tubular adenocarcinoma
  - Mucinous adenocarcinoma
  - Pooly cohesive carcinoma(including signet ring cell carcinoma and other variants)
  - Mixed carcinomas

- Adenosquamous carcinoma
- Carcinoma with lymphoid stroma(Medullary carcinoma)
- Hepatoid carcinoma

✓ Squamous cell carcinoma

✓ Undifferentiated carcinoma

NEUROENDOCRINE NEOPLASMS

- Neuroendocrine tumours (NET)
  - NET G1 (Carcinoid)
  - NET G2
- Neuroendocrine carcinoma (NEC)
  - Large cell NEC
  - Small cell NEC
- Mixed adenoneuroendocrine carcinoma
- EC cell, serotonin producing NET
- Gastrin producing NET (gastrinoma)

MESENCHYMAL TUMOURS

- Glomus tumours
- Granular cell tumour
- Leiomyoma
- Plexiform fibromyxoma
- Gastrointestinal stromal tumour
- Kaposi sarcoma
- Leiomyosarcoma
- Synovial sarcoma

Lymphomas

Secondary tumours

Diffuse type of gastric adenocarcinoma[26]

Popularly known as the linitis plastica. This carcinoma presents with diffuse thickening of the stomach wall. Mostly sporadic but 10% of the cases are inherited.

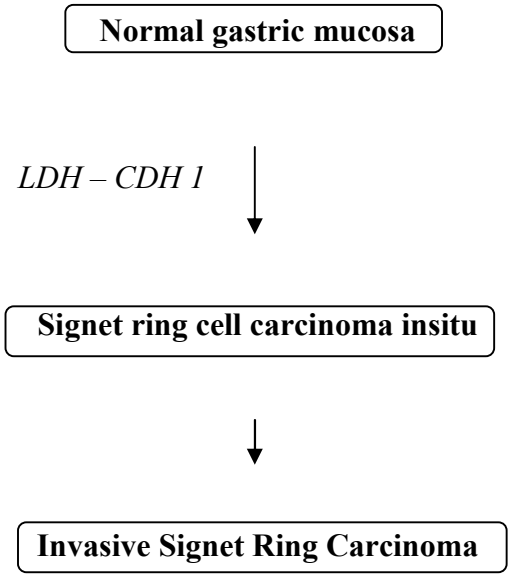
Hereditary diffuse gastric carcinoma :

It is due to dysregulation of three epithelial junctional proteins.They are involved in cellular interaction and adhesion.They is responsible for the development of hereditary diffuse type of gastric carcinoma. The three molecules are :

1. E. cadherin – A calcium dependent adhesion molecule.
2. Claudin – Tight junction proteins.
3. Connexions – Gap junction proteins.

Hereditary diffuse gastric cancer is inherited as an autosomal dominant condition. It occurs due to germline mutation in CDH1.Second hit mutation is necessary in these mutant carriers to initiate carcinogenesis.

Carneiro model of hereditary diffuse gastric cancer



Signet ring cell carcinoma in situ

Characterised by pagetoid spread of signet ring cells beneath foveolar epithelium. Glands & foveola have an intact basement membrane lined by signet ring cells. These cells have an eccentrically placed nucleus and mucous vacoules. These tumours are characterised by presence of low E- cadherin levels. Hence E –cadherin mutation is probably the earliest event in genesis of diffuse gastric Cancer.

Sporadic diffuse gastric cancer

Associated with H. pylori infection which induces methylation of the promoter region. There is associated silencing of E-cadherin. Methylation is reversible after H. pylori eradication. Diffuse gastric cancer is considered the most aggressive neoplasms. An exception to this is the desmoplastic variant that carries good prognosis

Tubular adenocarcinoma [4,7]

Composed of tubules of varying sizes.They showing branching, dilatation or slit like compression.Cells are cuboidal, columnar or may be flat due to intraluminal mucin.

Well differentiated

Glands of various sizes with minimal branching. The cells are either columnar or cuboidal. They have a pleomorphic basal nuclei, with thick nuclear margin and coarse chromatin.

Moderately differentiated

The glands are complex, irregular with marked architectural atypia. The tumour cells have a round irregular nuclei with loss of polarity and coarse chromatin.

Poorly differentiated

The cells are arranged in solid sheets and cords.There is sparse glandular elements. The nucleus is highly pleomorphic with numerous mitotic figures.

Papillary adenocarcinoma

These are well differentiated tumours composed of arborizing papillary projections. These projections have a core of fibrovascular connective tissue. These cores are lined by cuboidal cells that maintain polarity. The tumour shows clear demarcation with inflammatory cell infiltration.

Mucinous adenocarcinoma

Volume of mucin should be more than or equal to 50% of tumour volume. The tumour presents as small clumps of cells floating in pool of mucin. It can also present as glandular structures lined by mucin secreting cells.

Signet ring cell carcinoma

Tumour is composed of dispersed or small clusters of malignant cells. The cells can resemble a signet ring with nucleus pushed to one side due to intracytoplasmic mucin. They stain with alcian blue at pH 2.5 due to acidic mucin.

The Cells can also have varying morphology. Such as cells with centrally placed nucleus mimicking histiocytes, Neutral mucin containing small eosinophilic cells, Cells with scant mucin. The cells elicit a dense desmoplastic response.

Variants [6,35]

1)**Adenosquamous**

Rare tumour constituting <1%. Also called adenoacanthoma. The extent of squamous differentiation is variable. A number of hypothesis have been put forth regarding its pathogenesis. Most popular is the totipotent stem cells going for bidirectional differentiation. It is also believed to arise from metaplastic squamous epithelium.

Gross – Ulcerating large tumour

Microscopy

The squamous and the glandular element are intermingled. The squamous component should form >25% of tumour volume. The glandular element is predominantly of intestinal type with areas of metaplasia. The squamous component show varying degrees of differentiation.

Gastric carcinoma with a Lymphoid stroma [9,35]

First described in the Japanese population. Mimicks medullary carcinoma of breast. Affects cardia or the gastric stump. Common in men. Associated with EBV infection in 80% of cases.

Microscopy

Cellular lesion.Composed of sheets of cells with abundant cytoplasm, uniform nuclei with prominent nucleoli and coarse chromatin. The cells are PAS positive. Admixed with abundant lymphocytes that are predominantly CD8+ve T cells, plasma cells and giant cells. The giant cells are believed to formed due to interferon γ released by T cells.

Insitu hybridisation studies have demonstrated non polyadenylated RNA-1 (EBER). This is coded by EBV expressed in the nucleus of giant carcinoma cell.

Gastric carcinoma with extensive Neutrophilic infiltration[35]

Occurs commonly in women, associated with better outcome. Reported in anaplastic carcinoma with pleomorphic cells admixed with neuterophilic infiltration. It is due to the IL-8 produced by tumour cells.

Carcinoma with Rhabdoid feature[35]

Gross

Exophytic masses or ulcers.

Microscopy

Solid or poorly cohesive cells in diffuse pattern with minimal stroma. The individual cells have an eccentrically placed vesicular nuclei, abundant eosinophic cytoplasm with large inclusion.

IHC

Vimentin and cytokeratin positive. It is an aggressive carcinoma with bad prognosis.

Hepatoid and Alpha feto protein producing adenocarcinoma[5,39]

These tumours resemble hepatocellular carcinoma. Composed of polygonal cells with abundant granular eosinophilic cytoplasm. They are admixed with adenocarcinomatous components in either tubular or papillary pattern.

It is believed to develop due to the embryonal endodermal development towards liver and stomach.

Tendency to go for venous invasion and liver metastasis. Has a poor prognosis.

Special stains

The HCC component shows PAS positivity with diastase resistance in 50% of the tumour.

IHC

Positive for α feto protein, CEA, Albumin, α_1 antichymotrypsin.

TERATOMA [7]

Extremely rare, few cases have been reported in literature. Presents in infants with features of abdominal mass, obstructive symptoms and bleeding manifestation.

Gross

The tumour projects into the gastric lumen or into abdominal cavity.

Microscopy

Composed of elements from all three germ layers. Treatment is by surgical resection.

CHORIOCARCINOMA [7,35]

Cells positive for HCG are in the neck region of normal gastric glands. These are predominantly α subunits.

Clinical features

Can affect both sexes. An affected male often presents with Gynaecomastia .

Microscopy

Tumour is composed of malignant cytotropohoblast and syncitiotropoblasts. They are admixed with adenocarcinomatous component with varying differentiation.

Often admixed with embryonal carcinoma and yolk sac tumour. Patient has elevated serum β HCG levels. Placental lactogen and pregnancy specific glycoprotein may also be elevated.

IHC

Immuno reactive for α and β HCG.

CARCINOSARCOMA [7,35]

It is seen in young individuals, usually at pylorus. It carries a poor prognosis

Microscopy

Present as collision tumour with carcinomatous and sarcomatous elements meeting at interface. It also presents as composite tumour showing irregular admixture of both elements. The carcinomatous element is predominantly adenocarcinoma .The sarcomatous element shows differentiation towards rhabdomyosarcoma, leiomyosarcoma or chondrosarcoma.

IHC

The epithelial elements are positive for CEA, EMA and cytokeratin. The sarcomatous elements are positive for vimentin and desmin.

GASTRIC NEUROENDOCRINE NEOPLASMS[26]

The incidence of gastric carcinoid which was considered to be rare in the past is increasing. This is attributed to the increased endoscopic screening. These tumours are categorized based on their etiology as gastrin dependant (type I & II) and gastrin independent neoplasms (type III)

Type I gastric carcinoid

Occurs in the setting of atrophic gastritis and intrinsic factor deficiency. Associated with long term usage of proton pump inhibitors. Common in women in 5^{th} - 6^{th} decade.

Gross

Small lesions measure <1cm. Occur as multi centric polypoidal projections often located in the fundus. Confined to mucosa and submucosa. There is no vascular invasion.

Larger lesions measures > 1cm, either single or multiple. It is a low grade malignant neoplasm. Larger lesions have propensity for nodal and vascular involvement.

Microscopy

Entire spectrum starting from endocrine hyperplasia, adenomatoid hyperplasia, endocrine cell dysplasia and neoplasia may be present. They are well differentiated, benign grade I lesions with Ki-67 status <1%.

Molecular pathology

Loss of heterozygosity in 11q13-14 indicating involvement of MEN-1 gene. There is loss of methylation of LINE(1) [Long interspersed nucleotide element type 1]

Type II gastric carcinoids

It is associated with Zollinger Ellison syndrome, Diabetes, Hypothyroidism, MEN-1 syndrome and other endocrine abnormalities. These intermediate grade neoplasms present as multiple lesions of size <1cm.

Microscopy

They mimic type I lesion except for an increased tendency for local invasion and metastasis. They are well differentiated lesions with low or negligible malignant potential.

Molecular pathology

Loss of heterozygosity and germline mutation that disrupts Menin function.

Inactivates cell cycle inhibitors like $P16^{INK4A50}$ that results in loss of stimulus to inhibit cell proliferation.

Type III gastric carcinoids

These particular subsets of carcinoids are sporadic. Associated with normo-gastrinemic state. Occurs in men in 5^{th} - 6^{th} decade. They can be functioning resulting in atypical carcinoid syndrome or non functioning mimicking an adenocarcinoma.

Microscopy

Composed of round cells with uniform centrally placed nuclei. Has characteristic salt and pepper chromatin, inconspicuous nucleoli with occasional mitosis. The cells may assume a variety of architectural patterns. They form cords, ribbons with occasional rosette formation. These tumours are graded according to the mitotic activity and Ki-67 immuno-labelling

Grade	Mitotic activity	Ki-67 immunolabelling
G1	< 2mitosis / 10 HPf	<2 %
G2	2-20 mitosis / 10 HPf	3-20 %
G3	>20 mitosis / 10 HPf	>20 %

Type III carcinoids comprise small cell and large cell neuro endocrine carcinoma.

IHC

Positive for Chromogranin A, Synaptophysin, Serotonin, Pancreatic polypeptide, Histamine, Gastrin, Corticotropin, β-Melanocytic stimulatory hormone and Epinephrine.

Electron microscopy

Presence of dense core neuro secretory granules

Oncocytic Carcinoma[6]

The tumour is composed of solid sheets of cells. The cells have abundant granular eosinophilic cytoplasm.

Special stains: PTAH and Luxol fast blue

Electron microscopy: Numerous mitochondria, tubules, vesicles, intracellular canalicular system and microvilli filling the intercellular lumen.

Fundic Gland Adenocarcinoma [6]

It has predominantly chief cells. The cells have grayish blue cytoplasm and atypical nuclei. Commonly occur in proximal stomach and has good prognosis.

Gastric carcinoma with Osteoclast like giant cells[6]

The tumour cells are admixed with numerous multi nucleated giant cells.

Acinar cell carcinoma[6]

It is believed to arise from the region of pancreatic metaplasia.

Other rare variants[6]

Clear cell carcinoma

Micropapillary carcinoma.

GASTROINTESTINAL STROMAL TUMOUR[26]

These are spindle cell neoplasms that are CD117 positive. It is the first tumour to be used in the targeted therapy of group of drugs called Tyrosine kinase inhibitors.

Cell of origin: The gastric pace maker cells or the Interstitial cells of Cajal. They are present admixed with the myentric plexus of stomach.

Clinical features

Asymptomatic, may present with pain, bleeding manifestations and anemia. Common in people in the age group of 50-60 years. No known risk factors.

Gross

They can present as a dome shaped submucosal nodule or as a dumb bell shaped lesion with a narrowed segment in the muscularis propria and bulbous portion on both submucosa and serosa. Cut surface is firm to rubbery in consistency. Cut surface is grey white to yellow with areas of secondary degeneration.

Microscopy

The tumour assumes two pattern, the spindle cell type and epitheloid cell type. The spindle cell have a blunt elongated nucleus with a perinuclear cytoplasmic vacuole. They can assume varying pattern in forms of interlacing or interdigitating fascicles.

The epitheloid cell type is composed of sheets of cells with abundant cytoplasm, centrally placed round nucleus, prominent nucleoli & a perinuclear halo.

Prognostic feature

Include the tumour size and mitotic activity. Tumour <2cm and not more than 5 mitosis/50 HPf have a good prognosis.

Predictors of metastasis

Growth pattern, cellularity, type of cell, degree of proliferation and necrosis.

IHC

>95 % of the tumours are positive for KIT. The positivity can be membranous, cytoplasmic or a perinuclear dot.

Tumours that are KIT negative are positive for Anoctamin-1(Ano-1). It is a chloride channel protein in the cajal cells.Ano-1 is also called DOG-1 (Discovered on GIST) or ORAOV2 (Overexpressed in oral carcinoma)

Molecular genetics

Mutation in KIT or PDGFRA leads to activation due to self phosphorylation independent of ligand binding.

GASTRIC LYMPHOMA[5,21,23,26]

Lymphoid neoplasms of the stomach are classified into Hodgkins and Non-hodgkins lymphoma. The GIT is the most frequent site of occurrence of extranodal NHL.

The most common tumour in this category is Marginal Zone B Cell Lymphoma. The other tumours are Mantle cell lymphoma, Burkitt lymphoma, DLBCL, T cell lymphoma and Hodgkin's lymphoma.

Pathogenesis

The initiating stimulus in most cases is chronic H.pylori associated gastritis. It results in development of an organized lymphoid follicle in the gastric mucosa. These lymphoid follicles resemble the mucosa associated lymphoid tissue.It shows infiltration of the surface epithelium mimicking the intestinal Payer's patches. Infection with H.pylori causes neoplastic proliferation of cells in the mucosa associated lymphoid tissue.

Gross

Low grade lymphomas present as vague submucosal nodules. High grade lymphomas presents as exophytic mass associated with ulceration.

Microscopy

Composed of heterogenous population of centerocyte like cells, plasma cells and large lymphocytes. They are admixed with reactive lymphoid follicles. They are characterised by lymphoepithelial lesions. They are small groups of atypical lymphocytes invading the glands and causing destruction of the glandular epithelium. This feature may not always be present. Sometimes only few scattered epithelial cells are admixed with sheets of lymphoid cells. There is expansion of lamina propria by the lymphoid cells. It is necessary to examine the muscularis mucosa. It is not infiltrated by the usual lymphoid collections but do so in the presence of MALT lymphoma.

Diagnosis

The biopsies obtained by gastric mapping ensure adequate sampling of the gastric mucosa. Endoscopic ultrasound is recommended to predict the depth of invasion. The tumour is staged using Ann Arbor classification. The other alternative systems used are Paris staging system.

IHC

The neoplastic cells express pan B cell markers like CD20 and are negative for CD5, CD10, CD11c.

Molecular genetics

t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21), t(3;14)(p14.1;q32) they result in activation of NF-kB.

MESENCHYMAL TUMOURS[5]

Schwannoma

These are well circumscribed benign lesions. Occur in the middle aged individuals. Microscopically the tumour is composed of palisading spindle cells cuffed by lymphocytes.

IHC: Positive for S-100 and GFAP, negative for CD117, CD34, desmin, actin.

Leiomyoma / Leiomyosarcoma

These are rare circumscribed solitary neoplasms usually located in the cardia. Tumour is composed of spindle cells with a blunt edged nucleus and eosinophilic cytoplasm arranged in whorls and fascicles.

IHC

Positive for Actin and desmin.

Electron microscopy

Prominent basal lamina with plaque like sub plasmalemmal structures, bundles of microfilament and cell membrane based pinocytic vesicle.

Glomus tumours

The common site is antrum. Arise from pericapillary cells of glomus bodies. These tumours are composed of sheets of round cells with uniform nuclei and pale eosinophilic cytoplasm. May be admixed with hyalinized or myxoid areas.

Special stains

Pericellular stain pattern with reticulin.

Vascular tumours

Hemangiomas, Lymphangiomas, Hemangioendotheliomas, Kaposi sarcoma and Angiosarcomas are the vascular neoplasms occurring in stomach.

Gastroblastoma [35]

This rare biphasic tumour is composed of dual population of mesenchymal and epithelial cells. The sheets of bland spindle cells are admixed with small aggregates of epithelial cells. Both elements are bland.

Other rare tumours of stomach[21]

1) Synovial sarcoma
2) Plexiform fibromyxoma
3) Granular cell tumour.

Metastatic tumours to stomach

The common tumours to metastasize to stomach are lobular carcinoma breast, renal cell carcinoma, Melanoma, Hepatobiliary carcinomas. Distinctions from primary gastric tumours are mainly by usage of IHC.

SPREAD OF GASTRIC CARCINOMA:[6]

Carcinomas of the proximal stomach have a propensity to involve the lower oesophagus. Carcinomas of the distal stomach tend to involve the duodenum.

Tumours having an infiltrative pattern of growth are more prone to serosal involvement. They produce an advanced tumour stage. They can also involve adjacent organs such as spleen, pancreas,colon and omentum. Spread to the loco regional nodes occur via the mucosal and submucosal lymphatic plexus of stomach. Tumours produce deposits in perigastric, para aortic and coeliac nodes. Tumours of the distal third of the stomach involve the hepatoduodenal nodes. Infrequent sites of metastasis are lungs, ovaries and adrenal glands. Gastric adenocarcinomas also produce vascular invasion.

Krukenberg's tumour is the bilateral ovarian metastasis arising from diffuse gastric carcinoma. Intestinal type of gastric adenocarcinoma with ovarian metastasis simulate surface epithelial neoplasms. Cutaneous metastasis from gastric adenocarcinoma can resemble a primary adenexal tumour. Metastasis to the liver is more common in intestinal type of gastric adenocarcinoma. It spreads in a intra sinusoidal pattern. This results in hepatic failure. Diffuse type of gastric carcinoma involves the lungs and ovary.

TREATMENT OF GASTRIC CARCINOMA[6]

Surgical resection is the treatment of choice in gastric adenocarcinoma. The decision for gastrectomy is made largely on the basis of the extent of invasion of the gastric wall by the tumour proper. Gastrectomy is accompanied by lymph node dissection and splenectomy in some cases.

Total gastrectomy : Tumours involving the gastric cardia or higher up in the lesser or greater curvature. Carries the disadvantage of higher morbidity.

Sub total gastrectomy: Tumours of distal half of the stomach. The main disadvantage is that it carries a probability for recurrence in the remnant gastric stump.

Gastric carcinoma shows very little response to radiotherapy and chemotherapy.

PROGNOSTIC FACTORS IN GASTRIC CARCINOMA[6]

1. **Age of the patient**

   Gastric carcinoma occurring in younger individuals carries bad prognosis. This is attributed mainly to the late diagnosis. Also, there is increased incidence of diffuse type of gastric adenocarcinoma .

2. **Tumour stage**

The depth of penetration of the tumour into the serosa serves as an important factor in predicting the extent of metastasis. In individuals with serosal involvement, the surface area of the tumour involvement is also important in deciding the prognosis.

3. **Anatomical location**

Long term survival is higher in individuals who have tumours involving the distal half of the stomach. Survival rates are lowered in tumours of oesophagogastric junction,fundus and cardia.

4. **Margins of tumours**

Tumors with pushing borders carry a good prognosis when compared to those cases involving the stomach wall in a diffuse manner.

5. **Size of the tumour**

Tumours with small size carry a better prognosis when compared to larger tumours.

6. **Histological type and grading**

Lauren's intestinal type is said to be associated with better prognosis when compared to diffuse type of tumours. The Goseki classification system takes into account the extent of gland formation and content of intracellular mucin. It is said to be a better predictor of survival. Among the diffuse type of carcinomas better prognosis is attributed to the desmoplastic low grade tumours. Anaplastic high grade tumours having worse prognosis. Tumours in increasing order of malignancy are cohesive tumours of intestinal and solid type, diffuse tumours, mixed tumours, small cell neuroendocrine carcinoma and adenosquamous carcinoma.

7. **Inflammatory reaction**

Advanced stage tumours have abundant S 100 protein positive langerhans cells. Presence of inflammatory cell infiltrates in tumour interface and degenerative changes in tumours is associated with good prognosis.

8. Peri neural invasion

Tumours with perineural invasion are associated with bad prognosis.

9. Resected margins

Positive resected margins predict early tumour recurrence.

10. Regional lymph node involvement

Tumours with negative nodal involvement is said to have 50% 5 year survival rate. 5 year survival rate is <10% in tumours with lymph node metastasis. The number of nodes involved carries much prognostic significance than the staging. Micro metastasis in lymph nodes demonstrated by immunohistochemistry carries a prognostic significance.

11. Surgery performed

Survival rates are higher with Radical subtotal gastrectomy (22.1%). Radical lymphadenectomy is said to carry a good prognosis when compared to standard lymphadenectomy.

12. Molecular biology:

- The rate of cell proliferation determined using markers like P105, PC10 and DNA ploidy analysis using flow cytometry serves as an indicator of prognosis.
- Over expression of HER 2Neu is said to be one of the independent prognostic factor in gastric carcinoma.
- Increased expression of P53 is associated with decreased survival
- Increased levels of cathepsin is associated with poor survival and propensity for invasion and metastasis. Increased expression of $p27^{kip1}$ is associated with poor survival.

- Loss of FHIT protein is associated with poor prognosis.
- Increased expression of the T Antigen ,a precursor protein of blood MN system is associated with invasion and metastasis.

HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR/ HER2Neu[11,13,21,28,29,30]

HER2Neu also known as ErbB 2 is a 185 kilo dalton receptor. It is becoming popular in the past few years. It is considered a molecular target for targeted therapies in various carcinomas[27]. Knowledge on the molecular structure and functioning of this receptor is important. It helps us to understand its mode of action and therapeutic significance.

ErbB belongs to a family of tyrosine kinase receptors, that include four members ErbB1/HER1, ErbB2/HER2, ErbB3/HER3, ErbB4/HER4. They exert their influence in a number of steps of oncogenesis. They influence proliferation of cells, their survival, angiogenesis, cell migration invasion.[28,29]

The role of HER2 in tumorigenesis is due to its amplification. This abnormally overexpressed protein is associated with carcinomas of breast, stomach, oesophagus, colon, endometrium, cervix, ovary, lungs and the urothelium. The ErbB protooncogene on Ch17q21 codes for a transmembrane tyrosine kinase receptor.

This receptor has a functional intracellular domain of tyrosine kinase. It has a lipophilic membrane spanning domain with an extracellular ligand binding region.[29] The ligands for these receptors are members of EGF related peptide growth factor family.

They are produced as transmembrane precursors. They undergo proteolysis to produce soluble growth factors. These ligands are grouped into three categories.

The first group comprise receptors specifically binding to Erb B1- amphiregulin (AR), Transforming growth factor alpha (TGF-α). The second group of receptors show dual specificity to both ErbB1 and ErbB4, include betacellulin (BTC), heparin binding EGF (HB EGF) and epiregullin (EPR).

The third group of receptors are the neuregulins. They are further sub divided based on their binding affinity for both ErbB3 and ErbB4 (NRG 1 & NRG 2) or ErbB4 only (NRG 3 and NRG 4)[28].

Mechanism of Action[29]

Binding of ligand to the receptor results in their dimerisation, activation of cytoplasmic domain and phosphorylation. This process provides a docking site for the signaling molecules. This in turn results in the activation of RAS MAPK pathway. All this causes cell proliferation and inhibition of apoptosis via the PI-3K-AKT mTOR pathway.

Role of HER2/Neu[11]

The Neu proto oncogene is the rat homologue of HER2. It was first described when DNA from rats with neuroblastoma induced by a process of chemical carcinogenesis with nitrosoethyl urea was transfected to fibroblasts.

All members of EGFR family possess specific ligands except HER2. No particular ligand has been associated with HER2/Neu. This property may be attributed to a point mutation occurring in the base pair in position 2012 that replaces valine with glutamic acid. This mutation is localized to the transmembrane region that paves way for the activation of receptor without binding of a ligand.

Uniquness of HER 2/Neu [27]

Has no specific ligand and undergoes constitutional activation. This molecular behavior is responsible for the uncontrolled cell proliferation and increased cell survival in the absence of growth factors resulting in malignant transformation.

Role of HER2/Neu in Gastric Carcinoma

Gene amplification of HER2/Neu and its overexpression in gastric and gastrooesophageal carcinomas was first described in 1986[27]. The advent of targeted therapies in inoperable cancers had led to the therapeutic implication of HER 2/ Neu positive gastric carcinomas.[30]

Literature has reported varying positivity rates for HER2/Neu expression in gastric carcinomas. It ranges from 7-43%[13]. Initially the scoring system was based on the criteria for breast tumours but it resulted in a number of false negative results.

In order to avoid this under reporting a new grading system was devised for gastric carcinomas. This could be applied to cases diagnosed on both biopsies and gastrectomy specimens.

The disadvantage of using a biopsy material is that often an inadequate material may be obtained. Also exhaustion of tissues in the blocks prevents additional investigations. The biopsy obtained is often subjected to crush artifacts.

It has been recommended that due to tumour heterogenicity and nonspecific staining patterns a positive HER2 /Neu score of 2+ considered to be equivocal and is should be combined with ancillary techniques like fluorescent insitu hybridization to confirm the positivity.

Scores of 3+ is considered positive for HER2/Neu overexpression. Score of 0 and 1+ is considered negative.

Artifacts interfering with IHC scoring of HER2/Neu

1) Intestinal metaplasia

2) Edge and crush artifacts

3) Luminal staining

Amplification of HER2/Neu is said to have more association with intestinal type of carcinomas. These patients have lowered rates of survival. HER2/Neu is gaining the reputation as one of the independent prognostic markers in gastric carcinomas. It stands next only to the lymph node status in predicting the prognosis.[13]

The role of pathologist in confirming the immunoreactivity of the tumour is crucial. As with most of the drugs trastuzumab has its own disadvantage. The side effects of this drug include headache, nausea, fatigue, hypokalemia, hepatotoxicity, peripheral neuropathy, visual impairment, conjunctivitis.[7]

Ki -67 [12,32,33,34]

The term Ki 67 was coined from the German town of Kiel .Here an experiment was carried on 96 clones of mouse antibody. Ki 67 was the antigen that reacted with the clone numbered 67 and hence the name was derived.

The gene for Ki 67 is situated on chromosome10. Ki 67 is localized to the cell nucleus during the phase of G_1, **S** G_2 and mitosis of the cell cycle.It is related to ribosomal RNA transcription. It is a non histone protein that maintains the cell cycle. Ki 67 is predominantly located in the nucleus during the interphase. Later a majority of this protein moves to the surface of the chromosomes.

This antigen is not expressed in the resting cells of the G0 phase. Hence it is used to determine the extent of cellular proliferation in a given population of cells. It also determines the tumour aggressiveness.

Among the Ki 67 antibodies MIB1 is considered to be the most reliable marker in the proliferating cells. They are produced from myeloma cell line in mice fused with splenic cells of mice that has been immunized with Ki 67 antigen.

The other proliferation markers used in tumours are PCNA, AgNORs and techniques using incorporation of labeled nucleotide probes like titrated thymidine or bromodeoxyuridine. According to literature a remarkable co relation was obtained between expression of Ki 67 and the degree of tumour differentiation.

It was also shown to have a positive co relation with the TNM staging. In spite of this the role of Ki 67 in being considered an independent prognostic marker in gastric carcinomas is questionable.

MASTERCHART

S.No	PATH No	AGE	SEX	SPECIMEN	SITE	DIAGNOSIS	GRADE	Her 2	Ki 67
1	2041/12	50	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	MODERATE		
2	2067/12	55	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR (S)		
3	2080/12	62	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR		
4	2185/12	58	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
5	2220/12	40	MALE	BIOPSY	PYLORUS	ADENOCARCINOMA	MODERATE		
6	2315/12	65	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR	1+	60 %
7	2328/12	29	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
8	2435/12	65	MALE	BIOPSY	PYLORUS	ADENOCARCINOMA	MODERATE		
9	2532/12	62	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
10	2718/12	30	FEMALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
11	2800/12	62	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
12	2801/12	59	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR (S)		
13	2923/12	67	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
14	2935/12	45	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
15	2972/12	55	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
16	3043/12	54	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR		
17	3147/12	50	FEMALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
18	3217/12	39	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
19	3226/12	65	FEMALE	GASTRECTOMY	PYLORUS	MUCIN SECRETING ADENOCARCINO			

						MA			
20	3298/12	56	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
21	3307/12	65	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	MODERATE		
22	3405/12	55	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	MODERATE	0	20%
23	3619/12	53	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
24	3695/12	65	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	MODERATE		
25	3724/12	70	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	MODERATE		
26	3936/12	46	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
27	4092/12	50	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
28	4317/12	52	MALE	GASTRECTOMY	PYLORUS	ADENOCARCINOMA	MODERATE		
29	4352/12	63	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR		
30	4562/12	55	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR		

31	4563/12	50	MALE	GASTRECTOMY	PYLORUS	ADENOCARCINOMA	POOR		
32	4654/12	70	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
33	4723/12	46	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR		
34	4752/12	47	MALE	GASTRECTOMY	BODY	ADENOCARCINOMA	MODERATE		
35	4792/12	50	MALE	BIOPSY	BODY	ADENOCARCINOMA	POOR		
36	5001/12	54	MALE	GASTRECTOMY	BODY	MUCIN SECRETING ADENOCARCINOMA			
37	5060/12	60	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR		
38	72/13	54	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	MODERATE		
39	93/13	75	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	WELL		

40	178/13	65	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
41	268/13	75	FEMALE	BIOPSY	BODY	LOW GRADE DYSPLASIA			
42	296/13	55	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
43	350/12	35	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
44	408/13	65	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
45	505/13	50	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
46	542/13	60	MALE	BIOPSY	CARDIA	ADENOCARCINOMA	POOR		
47	588/13	60	MALE	GASTRECTOMY	DIFFIUSE GROWTH	ADENOCARCINOMA	POOR	0	60%
48	612/13	62	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
49	633/13	40	FEMALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
50	659/13	70	MALE	GASTRECTOMY	DIFFIUSE GROWTH	ADENOCARCINOMA	POOR (S)		
51	851/13	36	MALE	GASTRECTOMY	ANTRUM	MUCIN SECRETING ADENOCARCINOMA			
52	938/13	65	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR	1+	10%
53	961/13	60	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
54	1107/13	71	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR (S)		
55	1199/13	28	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR (S)		
56	1210/13	57	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR		
57	1315/13	53	MALE	BIOPSY	ANTRUM	LOW GRADE DYSPLASIA			
5	1397/1	5	FEMAL	GASTRECTO	ANTRU	ADENOCARCINO	MODERAT	2	30

8	3	5	E	MY	M	MA	E	+	%
59	1412/13	55	MALE	BIOPSY	BODY	ADENOCARCINOMA	WELL		
60	1495/13	50	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR		
61	1703/13	35	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
62	1931/13	55	FEMALE	BIOPSY	CARDIA	ADENOCARCINOMA	MODERATE		
63	2064/13	44	MALE	BIOPSY	PYLORUS	HIGH GRADE DYSPLASIA			
64	2508/13	45	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
65	2540/13	60	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR		
66	2568/13	55	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
67	2712/13	60	MALE	BIOPSY	PYLORUS	LOW GRADE DYSPLASIA			
68	2808/13	60	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR		
69	2889/13	60	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	MODERATE		
70	2925/13	60	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
71	2946/13	40	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	MODERATE		
72	2960/13	41	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR	0	30%
73	2978/13	50	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR		
74	2995/13	55	MALE	BIOPSY	BODY	ADENOCARCINOMA	POOR		
75	3062/13	60	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR(S)		
76	3118/13	75	MALE	GASTRECTOMY	BODY	ADENOCARCINOMA	WELL	0	10%
77	3152/13	50	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
78	3153/13	70	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
79	3182/13	50	FEMALE	BIOPSY	BODY	ADENOCARCINOMA	POOR		
8	3227/1	5	FEMAL	GASTRECTO	ANTRU	ADENOCARCINO	MODERAT	0	60

0	3	0	E	MY	M	MA	E		%
81	3265/13	50	MALE	GASTRECTOMY	PYLORUS	PAPILLARY ADENOCARCINOMA		3+	15%
82	3271/13	35	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	MODERATE	0	70%
83	3281/13	70	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR (S)	0	30%

84	3342/13	48	MALE	GASTRECTOMY	PYLORUS	EARLY GASTRIC CANCER	WELL		
85	3408/13	48	MALE	BIOPSY	BODY	ADENOCARCINOMA	POOR		
86	3474/13	70	FEMALE	BIOPSY	CARDIA	ADENOCARCINOMA	MODERATE		
87	3493/13	54	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
88	3494/13	60	MALE	BIOPSY	PYLORUS	ADENOCARCINOMA	MODERATE		
89	3514/13	77	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
90	3515/13	35	MALE	BIOPSY	BODY	ADENOCARCINOMA	WELL		
91	3576/13	53	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR		
92	3578/13	50	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
93	3597/13	65	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR (S)		
94	3605/13	45	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
95	3635/13	60	FEMALE	BIOPSY	BODY	ADENOCARCINOMA	POOR (S)		
96	3719/13	65	FEMALE	GASTRECTOMY	BODY	ADENOCARCINOMA	MODERATE	0	50%
97	3725/13	66	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
98	3733/13	62	MALE	BIOPSY	BODY	ADENOCARCINOMA	POOR		
99	3874/13	55	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
100	3894/13	60	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		

101	3965/13	65	MALE	BIOPSY	CARDIA	LOW GRADE DYSPLASIA			
102	4032/13	68	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
103	4124/13	55	MALE	BIOPSY	PYLORUS	ADENOCARCINOMA	MODERATE		
104	4140/13	45	MALE	BIOPSY	BODY	ADENOCARCINOMA	WELL		
105	4210/13	60	MALE	GASTRECTOMY	PYLORUS	ADENOCARCINOMA	WELL		
106	19/14	40	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
107	70/14	60	MALE	BIOPSY	BODY	HIGH GRADE DYSPLASIA			
108	181/14	60	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
109	230/14	65	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	WELL	0	15%
110	248/14	60	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	POOR(S)		
111	298/14	60	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	WELL	2+	50%
112	311/14	50	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
113	329/14	62	FEMALE	GASTRECTOMY	CARDIA	ADENOCARCINOMA	MODERATE	0	30%
114	511/14	52	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	WELL		
115	624/14	60	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	POOR	0	70%
116	760/14	70	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
117	771/14	50	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	WELL		
118	796/14	60	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
119	807/14	50	FEMALE	GASTRECTOMY	DIFFUSE GROWTH	ADENOSQUAMOUS			
120	810/14	40	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	WELL		
121	814/14	62	MALE	GASTRECTOMY	ANTRUM	MUCIN SECRETING ADENOCARCINO			

						MA			
122	864/14	70	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	WELL	1+	60%
123	880/14	52	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
124	957/14	74	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
125	974/14	60	MALE	GASTRECTOMY	BODY	ADENOCARCINOMA	WELL	0	70%
126	1002/14	44	FEMALE	GASTRECTOMY	DIFFUSE GROWTH	ADENOCARCINOMA	POOR	0	20%
127	1147/14	55	MALE	GASTRECTOMY	PYLORUS	ADENOCARCINOMA	WELL	3+	40%
128	1160/14	52	FEMALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	WELL	0	10%
129	1195/14	40	MALE	BIOPSY	ANTRUM	LOW GRADE DYSPLASIA			
130	1203/14	52	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	WELL	1+	20%
131	1237/14	35	MALE	GASTRECTOMY	ANTRUM	ADENOCARCINOMA	WELL	0	20%
132	1356/14	60	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	WELL		
133	1381/14	61	MALE	BIOPSY	BODY	ADENOCARCINOMA	MODERATE		
134	1401/14	55	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	WELL		
135	1414/14	45	MALE	GASTRECTOMY	DIFFUSE GROWTH	ADENOCARCINOMA	POOR	0	10%
136	1491/14	45	FEMALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		
137	1505/14	62	MALE	GASTRECTOMY	PYLORUS	ADENOSQUAMOUS			
138	1698/14	50	FEMALE	GASTRECTOMY	PYLORUS	MUCIN SECRETING ADENOCARCINOMA			
139	1735/14	45	FEMALE	GASTRECTOMY	PYLORUS	ADENOCARCINOMA	MODERATE	0	50%
140	1903/14	75	MALE	BIOPSY	ANTRUM	ADENOCARCINOMA	MODERATE		

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

I. INCIDENCE OF GASTRIC CARCINOMAS :

This study was carried out in the Department Of Pathology Thanjavur Medical College From July 2012 to June 2014. The specimens were received from the Department of Surgery and Gastroenterology. A total of 260 specimen including both endoscopic biopsies and gastrectomy Specimens were received.

Benign lesions such as tissues with normal study, chronic gastritis, ulcers, Non neoplastic polyps,intestinal metaplasia, inadequate material, and lesions within 5cm from the oesophagogastric junction where excluded from this study. The remaining 140 malignant lesions were included in the study. They included 50 Gastrectomy specimens and 90 Endoscopic biopsies.

TABLE 1 : INCIDENCE OF GASTRIC CARCINOMAS AMONG OTHER CARCINOMAS IN THANJAVUR MEDICAL COLLEGE

S.NO	TIME PERIOD	TOTAL NO.OF MALIGNANCIES	NO. OF GASTRIC CARCINOMAS
1.	JULY 2012 – DEC 2012	525	37
2.	JAN 2013 – JUNE 2013	550	25
3.	JULY 2013 – DEC 2013	502	43
4.	JAN 2014 – JUNE 2014	488	35
TOTAL		2065	140
INCIDENCE RATE OF GASTRIC CARCINOMAS - 6.78% per 1000 malignancies			

CHART 1 : INCIDENCE OF GASTRIC CARCINOMA IN THIS STUDY

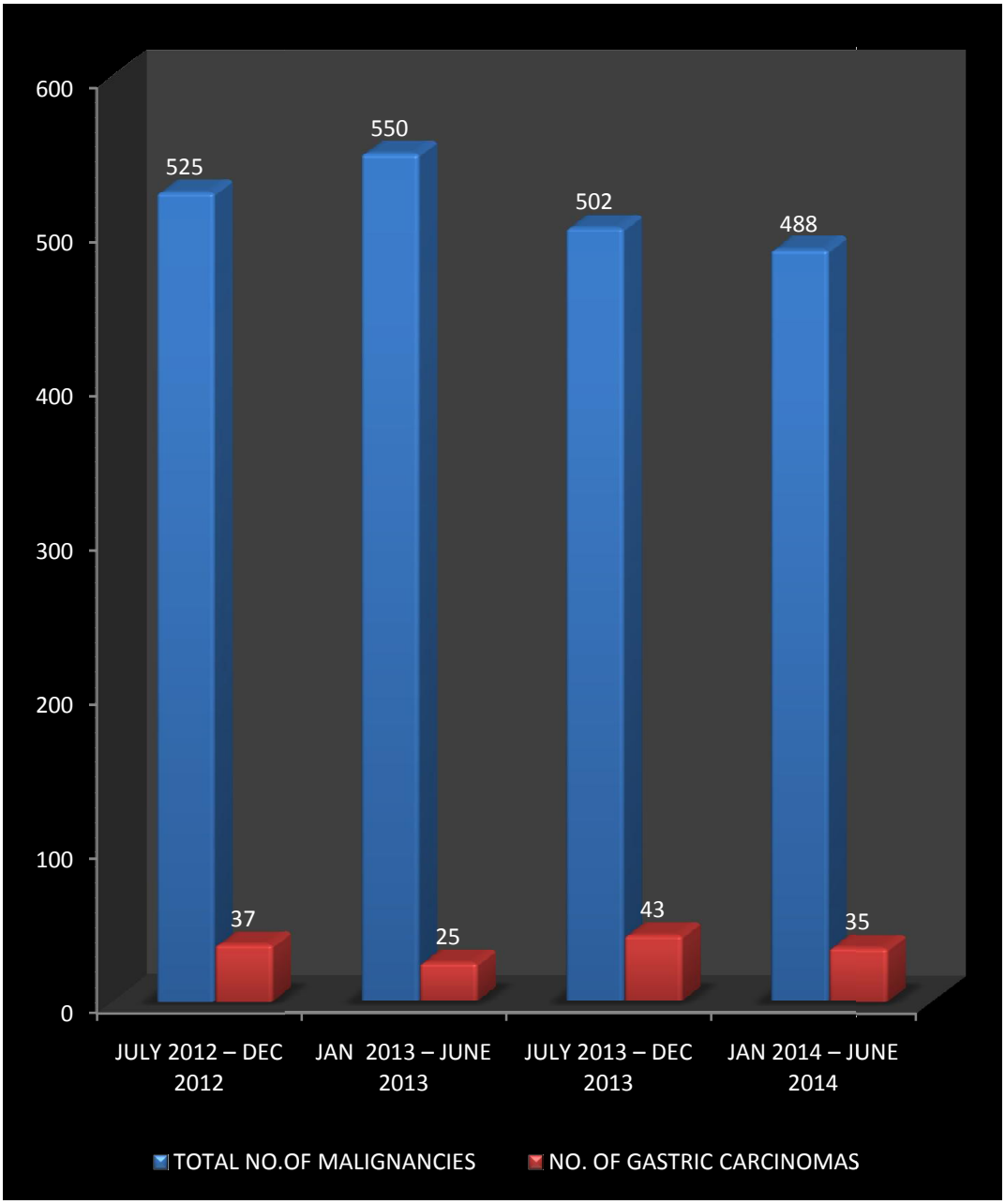


TABLE 2 : YEARLY INCIDENCE OF GASTRIC CANCER IN THANJAVUR MEDICAL COLLEGE

S.NO	TIME PERIOD	NO. OF CASES
1.	JULY 2012 – DEC 2012	37
2.	JAN 2013 – JUNE 2013	25
3.	JULY 2013 – DEC 2013	43
4.	JAN 2014 – JUNE 2014	35

CHART 2 : YEARLY INCIDENCE OF GASTRIC CANCER IN THANJAVUR MEDICAL COLLEGE

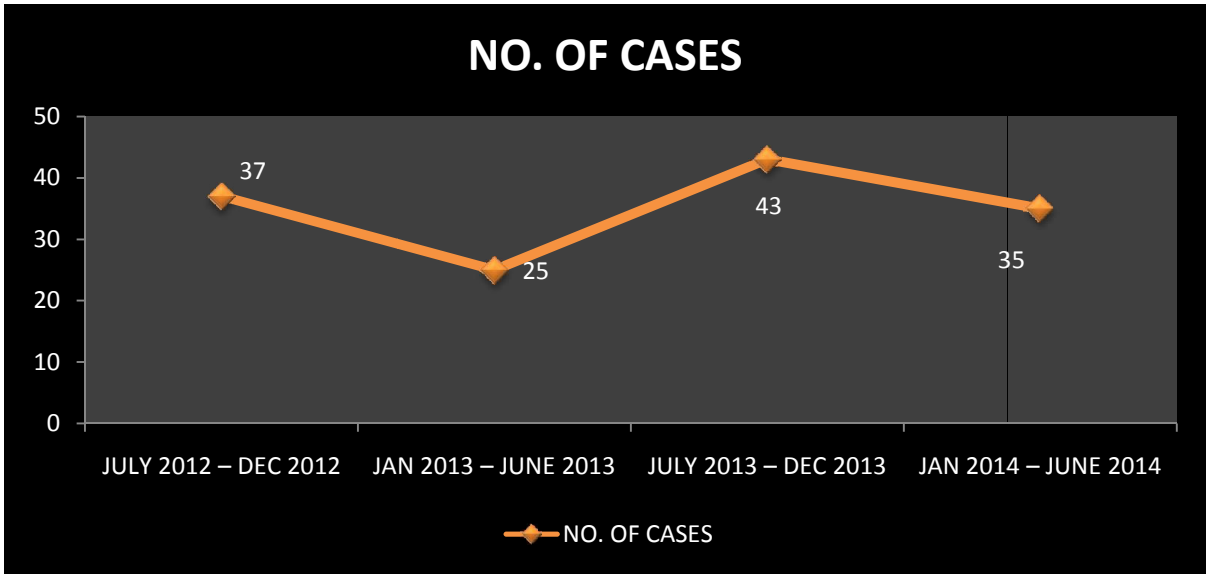


TABLE 3: AGE WISE INCIDENCE OF GASTRIC CARCINOMA IN THANJAVUR MEDICAL COLLEGE

S. NO	AGE	NO. OF CASES
1.	20 -29	1
2.	30 -39	9
3.	40 -49	20
4.	50 – 59	48
5.	60 -69	46
6.	>70	16
TOTAL		140

Of the total number of malignancies the age group that was most affected is people in their fifth and sixth decade of life. The maximum number of cases 48 was observed in the age group 50 -59. This is closely followed by 46 cases in individuals in age group of 60 -69. The incidence was the least in individuals below the age group of 30 years.

CHART 3 : AGE WISE INCIDENCE OF GASTRIC CARCINOMAS

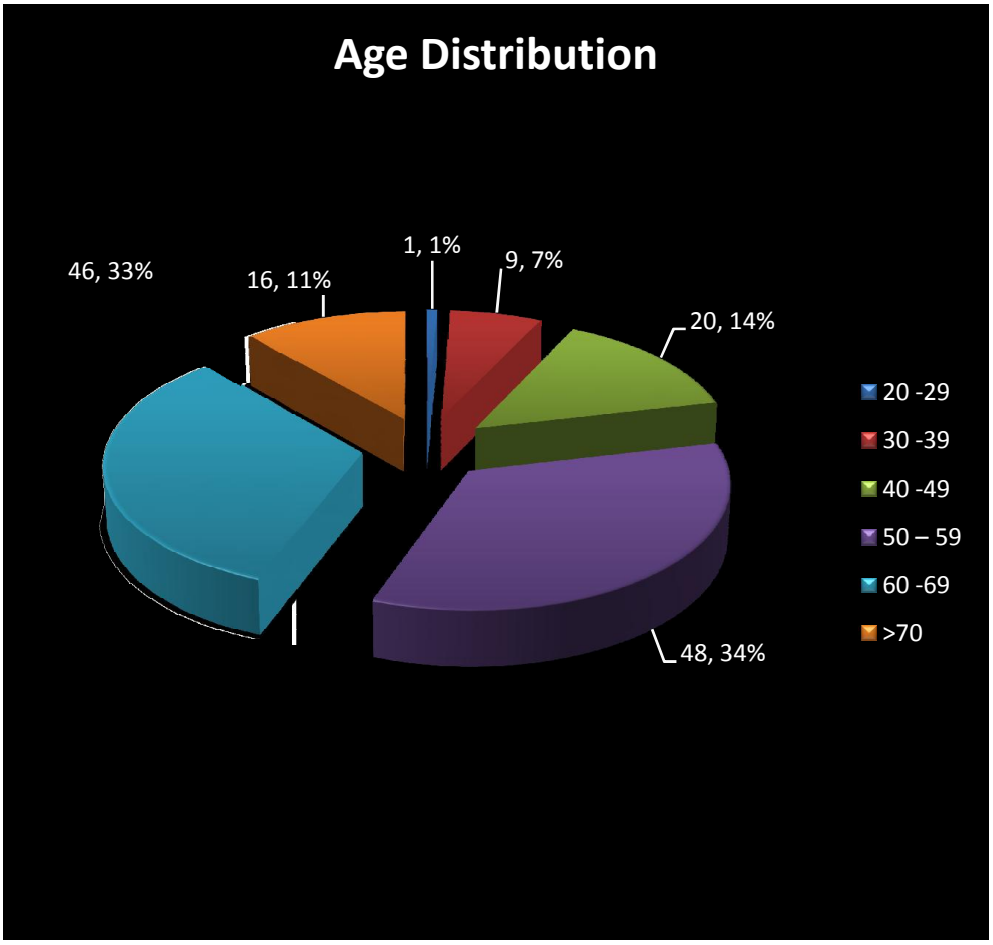
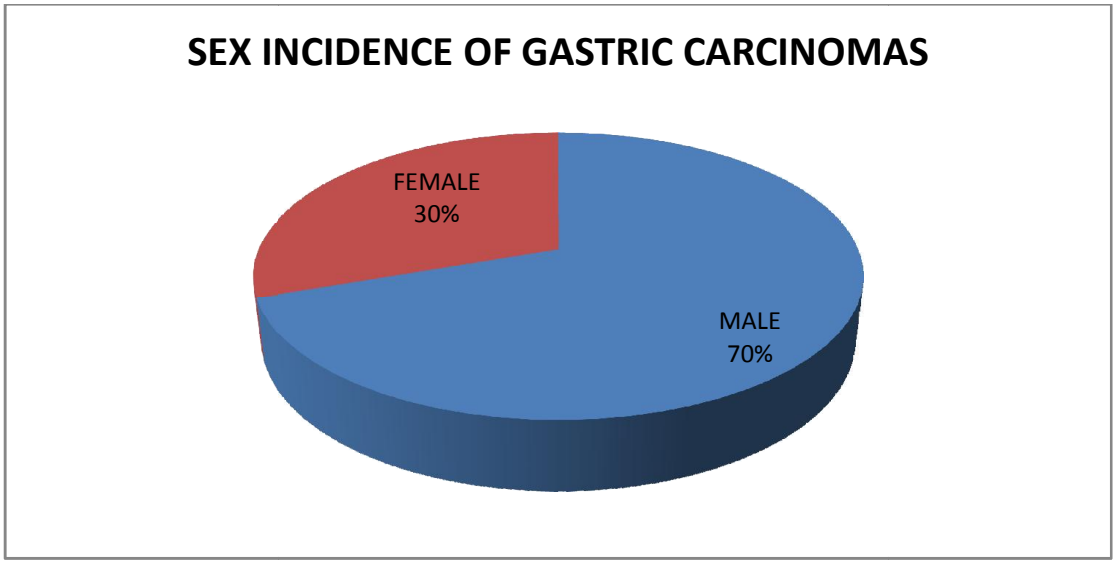


TABLE 4 : SEX WISE INCIDENCE OF GASTRIC CARCINOMA

TOTAL CASES	MALE	FEMALE
140	101	39

CHART 4 : SEX WISE INCIDENCE OF GASTRIC CARCINOMA



Among the total cases of gastric carcinoma 102 cases were of the male sex constituting 70% . The females cases formed the remaining 30% with 39 cases.

TABLE 5 : LESIONS IN ENDOSCOPIC BIOPSY SPECIMENS MALE

S.NO	LESION	CASES
1.	CHRONIC GASTRITIS	12
2.	ULCER	18
3.	INTESTINAL METAPLASIA	1
4.	DYSPLASIA	6
5.	CARCINOMA	58
6.	NORMAL STUDY	24
7.	NIL TISSUE	8
TOTAL		**127**

Of the 127 endoscopic biopsy samples from male patients 58 cases were positive for carcinoma. 24 cases showed normal study. 18 cases were reported as ulcer with granulation tissue. 12 cases were reported as chronic gastritis. 8 samples were inadequate. 6 cases showed dysplastic changes.

CHART 5 : ENDOSCOPIC BIOPSIES IN MALE

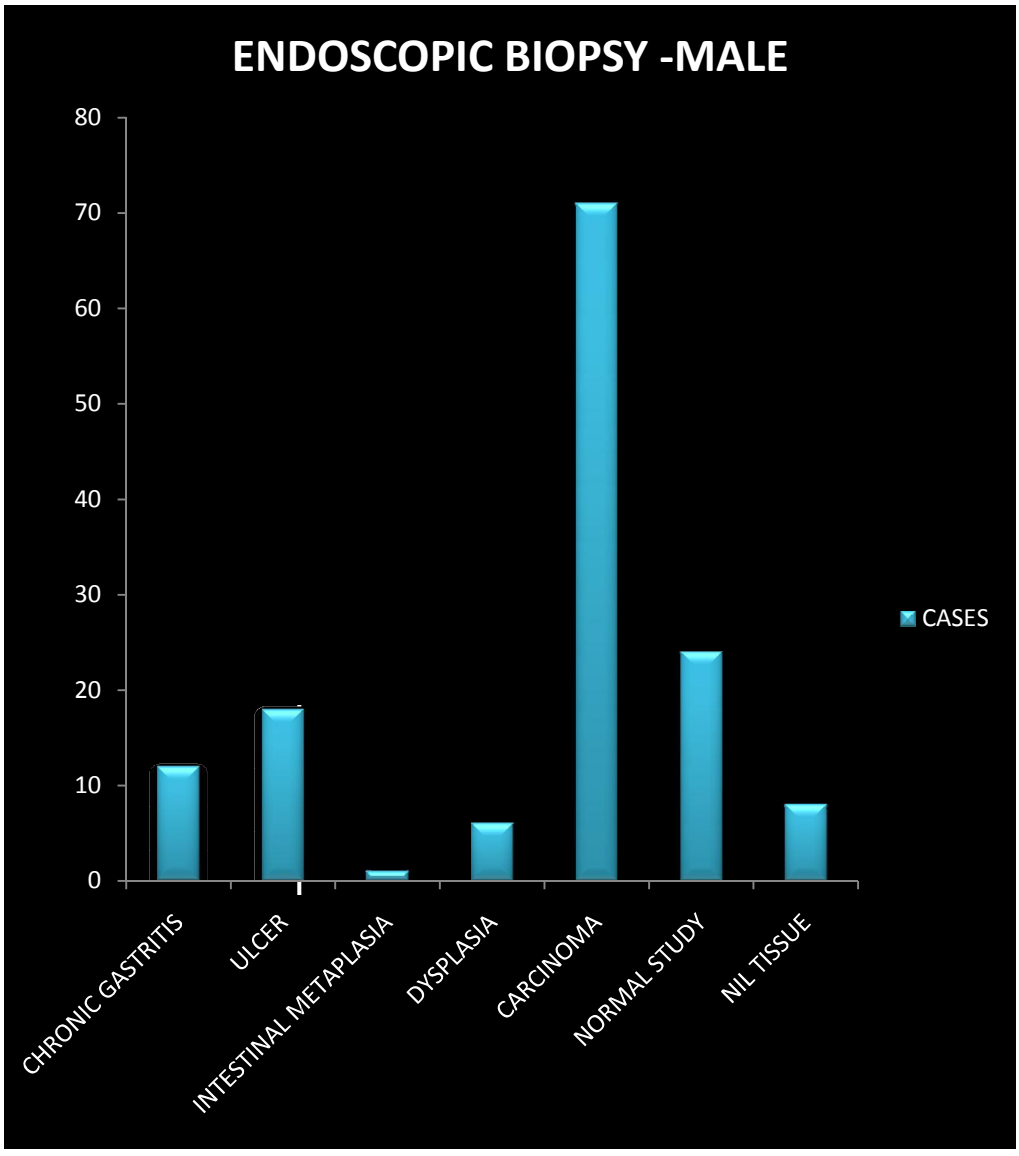


TABLE 6 : LESIONS IN ENDOSCOPIC BIOPSY FEMALE

S. NO	LESIONS	CASES
1.	CHRONIC GASTRITIS	7
2.	ULCER	9
3.	INTESTINAL METAPLASIA	1
4.	DYSPLASIA	1
5.	CARCINOMA	18
6.	NORMAL STUDY	5
7.	NIL TISSUE	4
	TOTAL	45

Of the 45 endoscopic biopsy samples from female cases 18 showed malignancy. 7 cases were reported as chronic gastritis. 9 cases were ulcer. 5 cases showed normal histology. 4 samples were inadequate. 1 case showed dysplastic changes.

CHART 6: LESIONS IN ENDOSCOPIC BIOPSY FEMALE

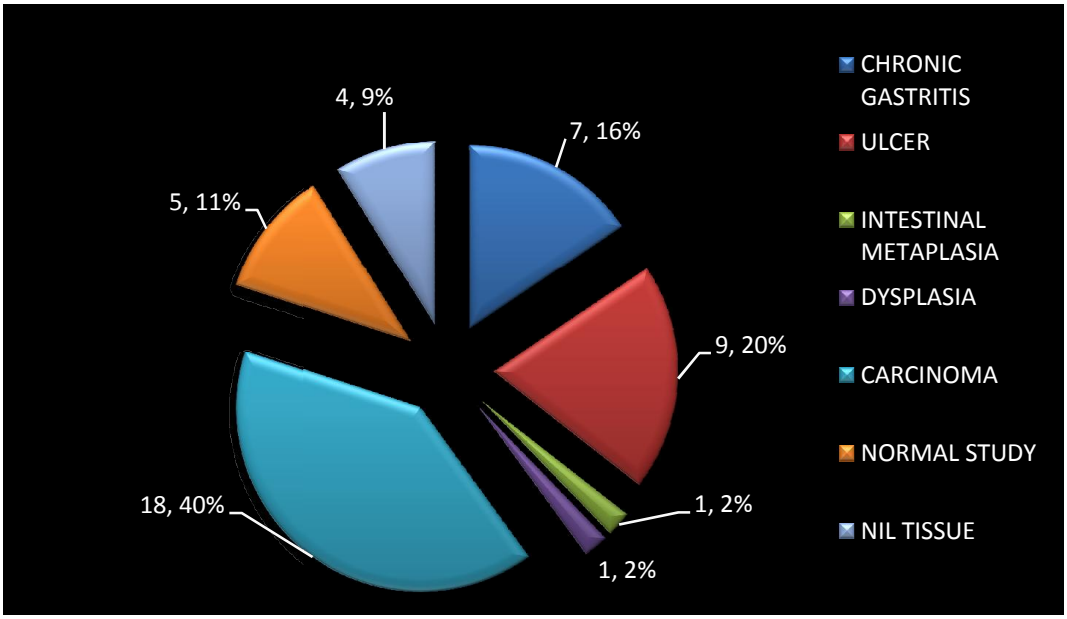


TABLE 7 : GASTRIC CARCINOMAS IN ENDOSCOPIC BIOPSIES AND GASTRECTOMY SPECIMENS

PROCEDURE	NO. OF CASES
ENDOSCOPIC BIOPSY	90
GASTRECTOMY	50

Of the total samples studied 50 were gastrectomy specimens. This included partial, subtotal and total gastrectomies. 90 were endoscopic biopsies.

CHART 7: GASTRIC CARCINOMAS IN ENDOSCOPIC BIOPSIES AND GASTRECTOMY SPECIMENS

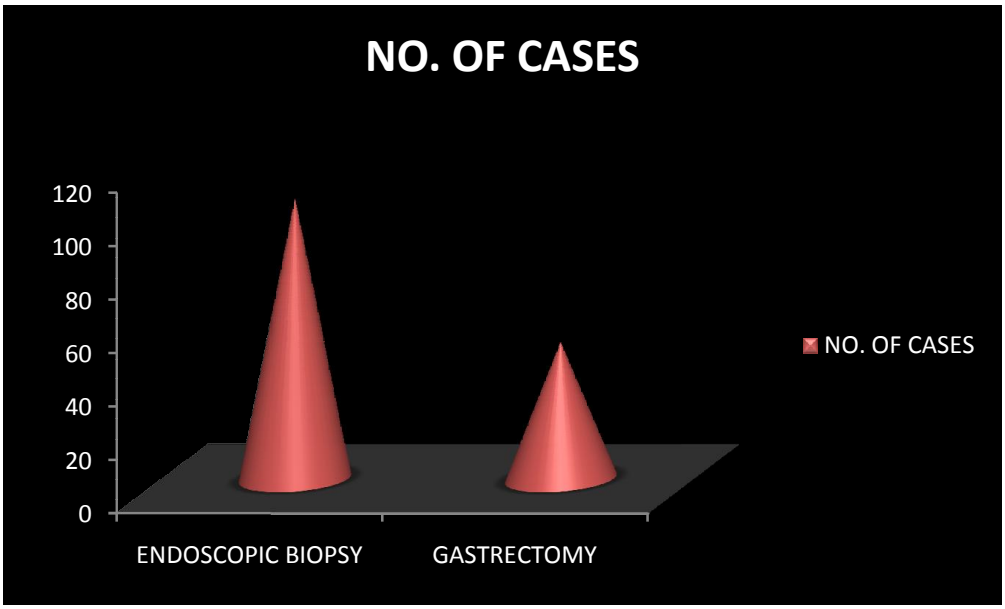


TABLE 8 : LOCATION OF GASTRIC CARCINOMAS

LOCATION	NO. OF CASES
CARDIA	5
BODY	30
ANTRUM	84
PYLORUS	16
DIFFUSE GROWTH	5

In this study it was observed that most of the malignant tumours occurred in the antrum with a total of 84 cases constituting around 60 % followed by the body of the stomach with 30 Cases. The occurance of tumours in the proximal stomach is the least with only 5 cases forming 3.57%. Hence the occurance of malignant tumours is more in the distal region when compared to the proximal regions of stomach.

CHART 8: LOCATION OF GASTRIC CARCINOMAS

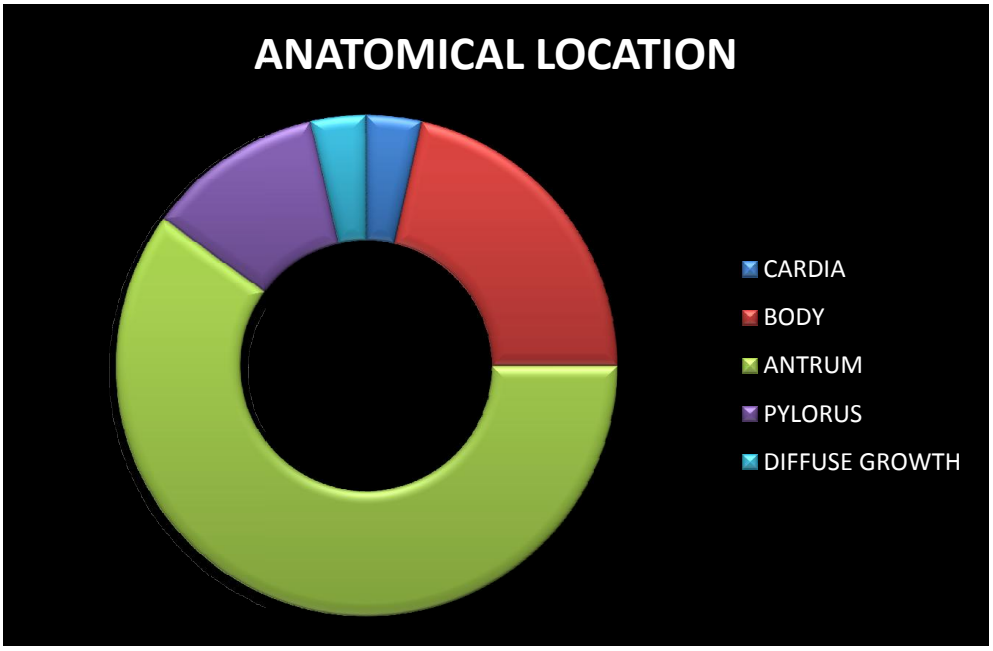


TABLE 9 : CLASSIFICATION OF GASTRIC CARCINOMA ACCORDING TO DIFFERENTIATION

WELL	MODERATE	POOR
20	69	37

Majority of cases were reported as moderately differentiated adenocarcinoma with a total of 69 cases. 37 cases were poorly differentiated. 20 cases were well differentiated.

CHART 9 :CLASSIFICATION OF GASTRIC CARCINOMA ACCORDING TO DIFFERENTIATION

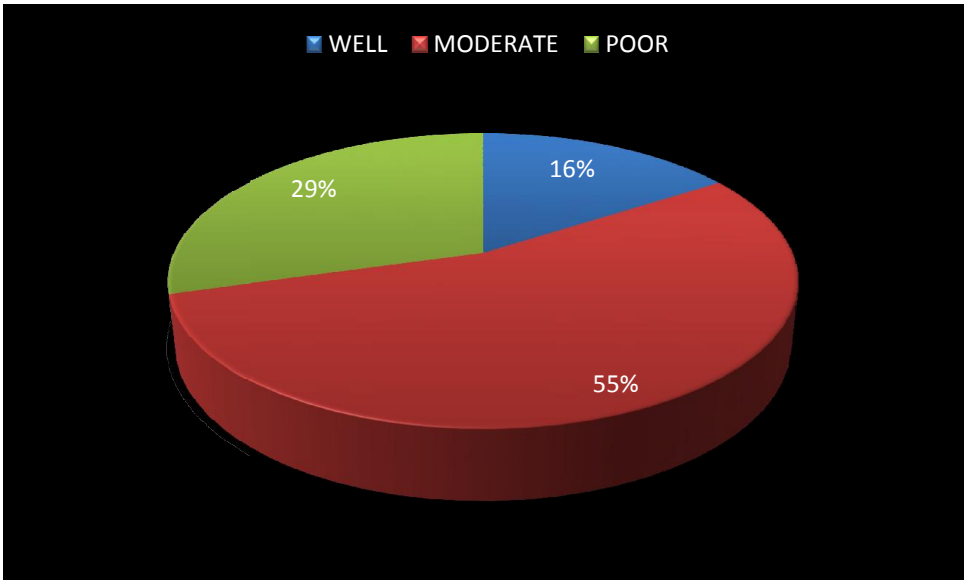


TABLE 10 : W.H.O. CLASSIFICATION OF GASTRIC CARCINOMAS

S.NO	TUMOUR TYPE	NO. OF CASES
1.	TUBULAR ADENOCARCINOMA	117
2.	PAPILLARY ADENOCARCINOMA	1
3.	MUCINOUS ADENOCARCINOMA	5
4.	POORLY COHESIVE CARCINOMA(SIGNET RING)	8
5.	ADENOSQUAMOUS CARCINOMA	2
6.	LOW GRADE DYSPLASIA	5
7.	HIGH GRADE DYSPLASIA	2

In this study 117 cases were reported as Tubular adenocarcinoma. 5 cases as mucinous adenocarcinoma. 8 cases were reported as poorly cohesive carcinoma containing signet ring cells. 1case of papillary adenocarcinoma and 2 cases of adenosquamous carcinoma were reported

CHART 10 : CLASSIFICATION ACCORDING TO W.H.O. CRITERIA

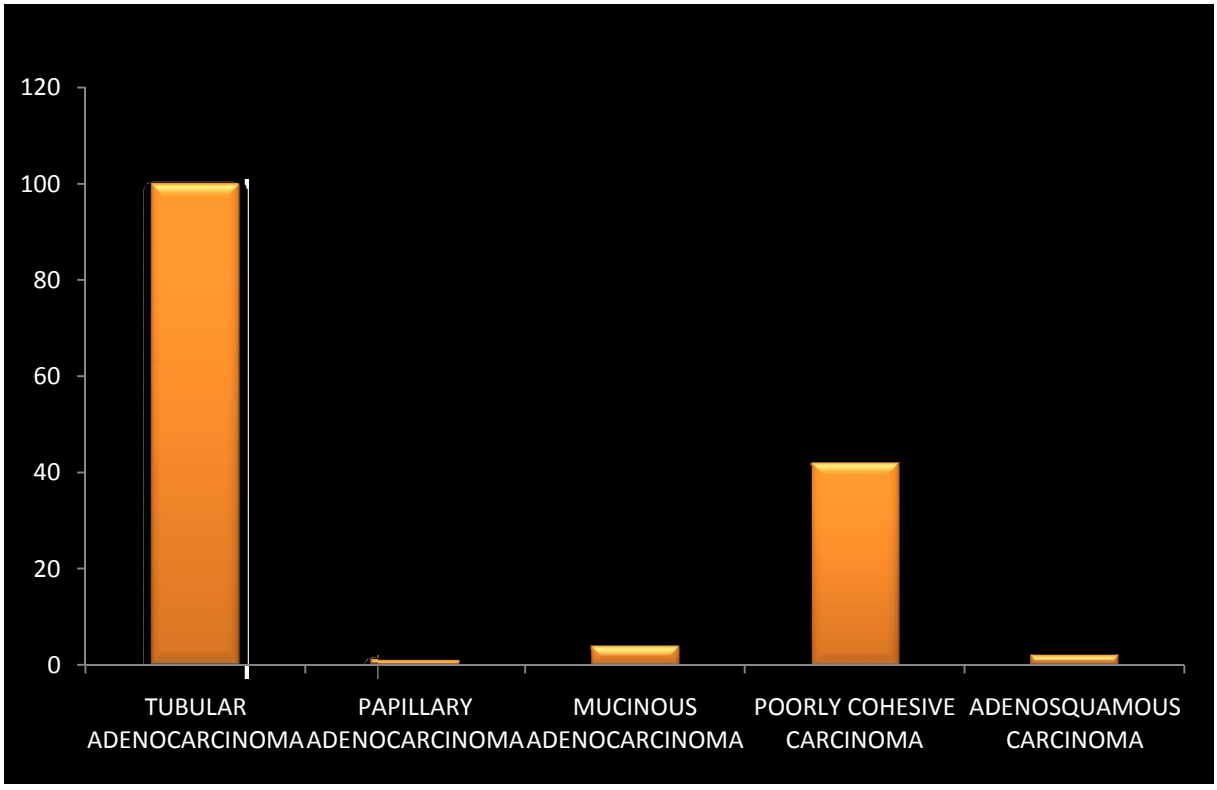


TABLE 11 : CLASIFICATION OF GASTRIC CARCINOMAS ACCORDING TO LAUREN'S CLASSIFICATION

LAUREN'S CLASSIFICATION	NO. OF CASES
INTESTINAL	125
DIFFUSE	8

Of the total cases 125 cases were reported as intestinal type of adenocarcinoma & 8 cases were reported as diffuse type of carcinoma according to the Lauren's classification.

CHART 11 : CLASSIFICATION OF GASTRIC CARCINOMAS ACCORDING TO LAUREN'S CLASSIFICATION

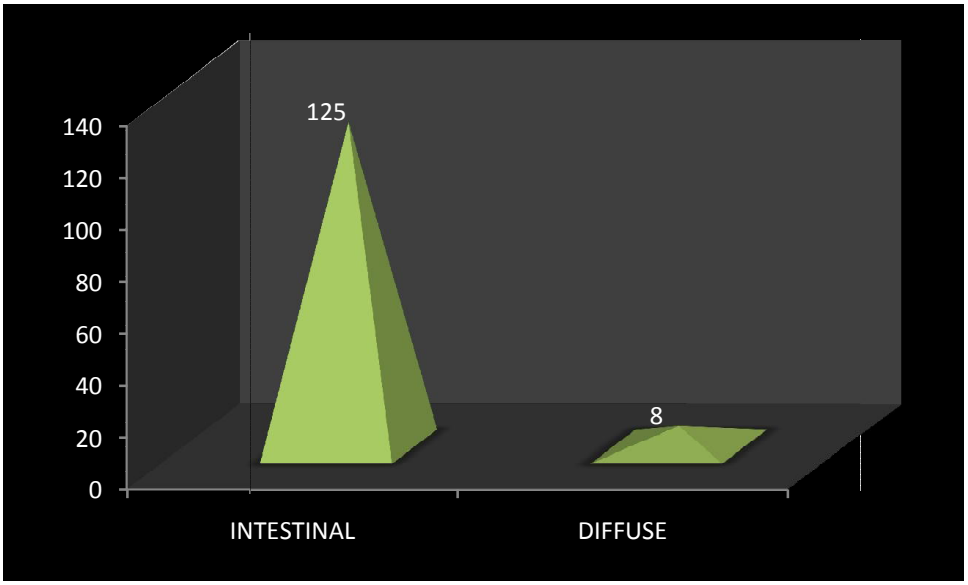


TABLE 12 : EXTENT ON INVASION BASED ON TNM STAGING

S.NO	EXTENT OF PRIMARY TUMOUR(T)	NO. OF. CASES
1.	T1 a	1
2.	T1 b	9
3.	T2	18
4.	T3	8
5.	T4 a	13
6.	T4 b	1

In this study 18 cases showed tumour invasion of muscularis propria constituting 36%, followed by 13 cases showing invasion into the visceral peritoneum constituting 26%. Early gastric carcinomas formed 20% of cases with a total of 10 cases. One case showed tumour involving the adjacent structures (2%) (Appendix IV)

CHART 12: EXTENT ON INVASION BASED ON TNM STAGING

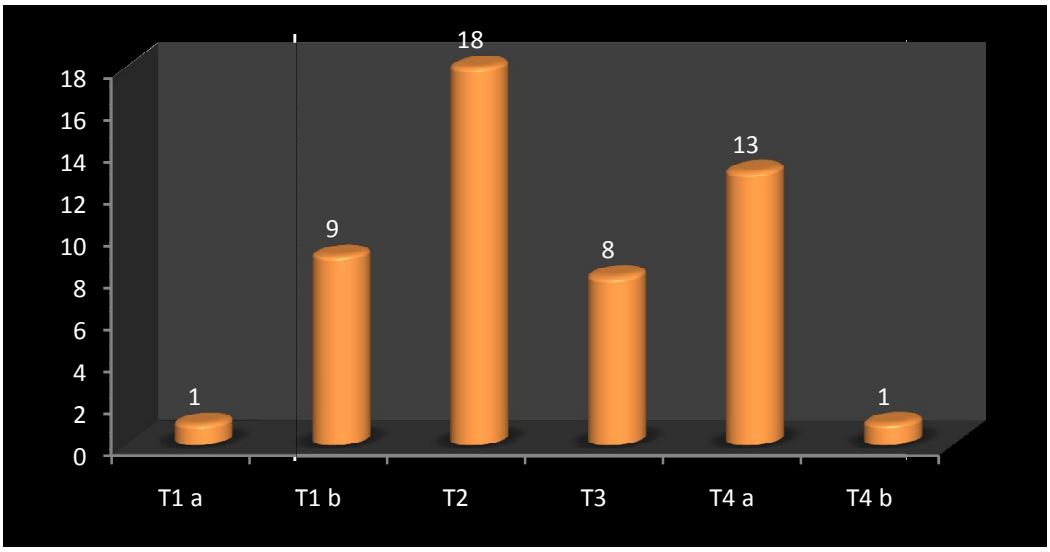
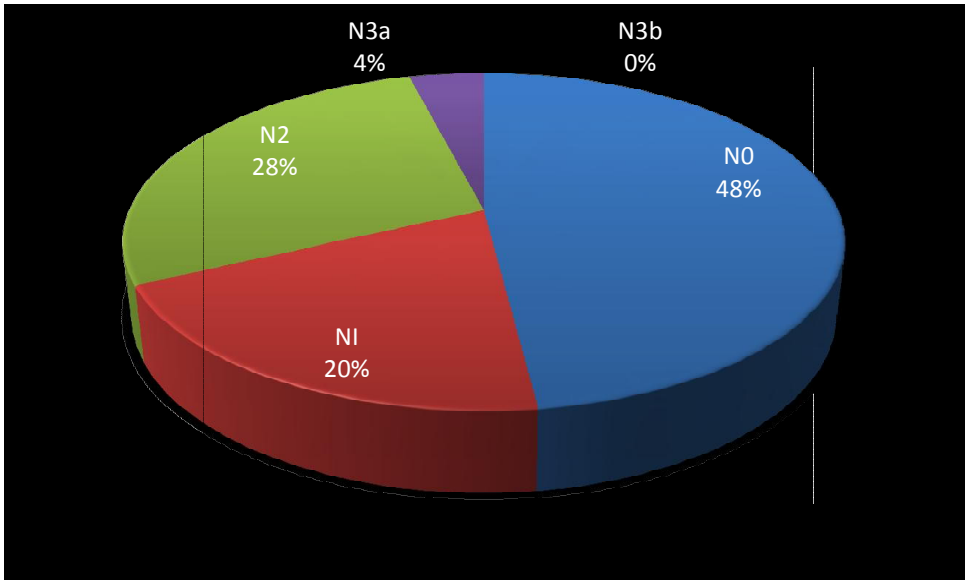


TABLE 13: LYMPH NODE STATUS BASED ON TNM CLASSIFICATION

S. NO	LYMPH NODE STATUS	NO. OF CASES
1.	N0	24
2.	NI	10
3.	N2	14
4.	N3a	2
5.	N3b	0

In this study the majority of cases showed no nodal involvement , namely 24 cases(48%), Closely followed by cases with N2 status, 14 cases (28%). 20% of the tumours showed N1 Status and 2 cases (4%) are of N3a status, none of the cases were positive for N3b. (Appendix IV)

CHART 13 : LYMPH NODE STATUS BASED ON TNM CLASSIFICATION



IMMUNOHISTOCHEMISTRY – HER 2 EXPRESSION

S.NO	PATH No.	GRADE OF DIFFERENTIATION	HER 2 GRADE
1.	2315/12	POORLY DIFFERENTIATED ADENOCARCINOMA	1+
2.	3405/12	MODERATELY DIFFERENTIATED ADENOCARCINOMA	0
3.	588/13	POORLY DIFFERENTIATED ADENOCARCINOMA	0
4.	938/13	POORLY DIFFERENTIATED ADENOCARCINOMA	1+
5.	1397/13	MODERATELY DIFFERENTIATED ADENOCARCINOMA	2+
6.	2960/13	POORLY DIFFERENTIATED ADENOCARCINOMA	0
7.	3118/13	WELL DIFFERENTIATED ADENOCARCINOMA	0
8.	3227/13	MODERATELY DIFFERENTIATED ADENOCARCINOMA	0
9.	3265/13	PAPILLARY ADENOCARCINOMA	3+
10.	3271/13	MODERATELY DIFFERENTIATED ADENOCARCINOMA	0
11.	3281/13	SIGNET RING CELL CARCINOMA	0
12.	3719/13	MODERATELY DIFFERENTIATED ADENOCARCINOMA	0
13.	230/14	WELL DIFFERENTIAED ADENOCARCINOMA	0
14.	298/14	WELL DIFFERENTIATED ADENOCARCINOMA	2+
15.	329/14	MODERATELY DIFFERENTIATED ADENOCARCINOMA	0

16.	624/14	POORLY DIFFERENTIATED ADENOCARCINOMA	0
17.	864/14	WELL DIFFERENTIATED ADENOCARCINOMA	1+
18.	974/14	WELL DIFFERENTIATED ADENOCARCINOMA	0
19.	1002/14	POORLY DIFFERENTIATED ADENOCARCINOMA	0
20.	1147/14	WELL DIFFERENTIATED ADENOCARCINOMA	3+
21.	1160/14	WELL DIFFERENTIATED ADENOCARCINOMA	0
22.	1203/14	WELL DIFFERENTIATED ADENOCARCINOMA	1+
23.	1237/14	WELL DIFFERENTIATED ADENOCARCINOMA	0
24.	1414/14	POORLY DIFFERENTIATED ADENOCARCINOMA	0
25.	1735/14	MODERATELY DIFFERENTIATED ADENOCARCINOMA	0

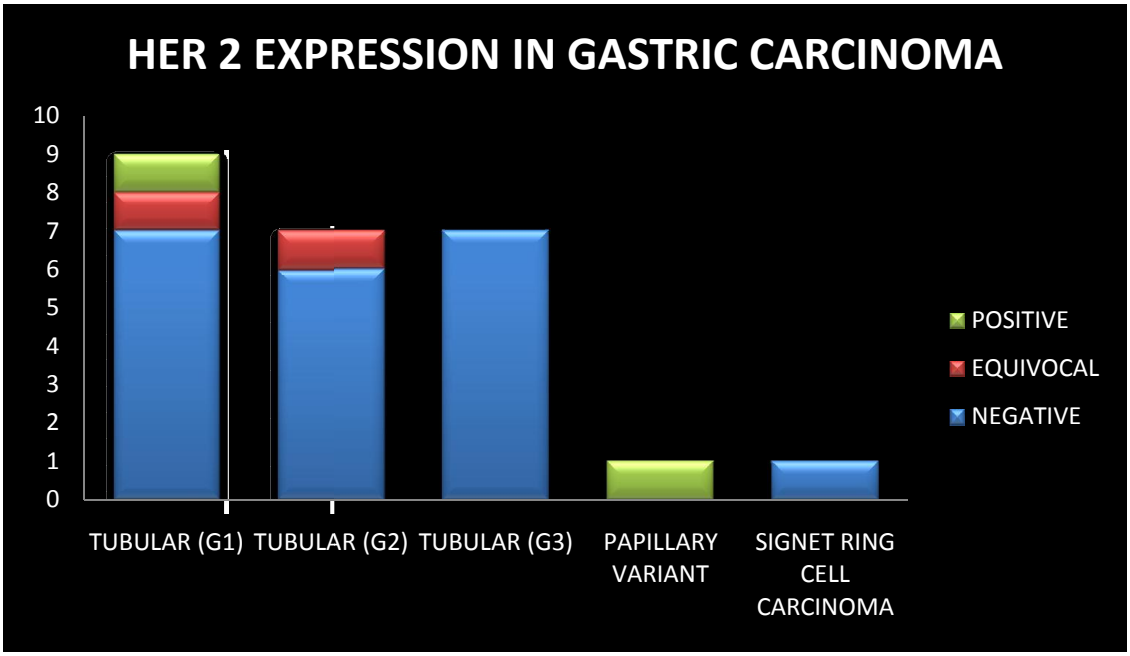
25 cases in this study were chosen for immunohistochemical analysis. This included gastrectomy specimens and reported as tubular adenocarcinoma, papillary adenocarcinoma and signet ring cell carcinomas. IHC grading of 0 and 1+ is considered negative, 2+ is considered equivocal and 3+ is considered positive. **Chi square =2.701 p>0.05. Not significant. There is no association between the grade and HER2 levels.**

TABLE 14 : HER 2 EXPRESSION IN VARIANTS OF GASTRIC CARCINOMA

S.NO	TYPE OF CARCINOMA	NO. OF CASES	NEGATIVE	EQUIVOCAL	POSITIVE
1.	TUBULAR (G1)	9	7	1	1
2.	TUBULAR (G2)	7	6	1	0
3.	TUBULAR (G3)	7	7	0	0
4.	PAPILLARY VARIANT	1	0	0	1
5.	SIGNET RING CELL CARCINOMA	1	1	0	0

In this study out of 25 cases only 2 cases showed 3+ positivity (8%) , 2 cases showed equivocal grading (8%) and 21 cases showed negative results with score of 1+ or 0 (84%) . Tumours with signet ring cell morphology showed a negative scoring.

CHART 14 : IMMUNOHISTOCHEMISTRY – HER 2



IMMUNOHISTOCHEMISTRY- KI 67

S.NO	PATH NO	AGE/ SEX	GRADE OF DIFFERENTIATION	Ki 67 Score
1.	2315/12	65/M	POORLY DIFFERENTIATEDADENOCARCINOMA	60%
2.	3405/12	55/M	MODERATELY DIFFERENTIATED ADENOCARCINOMA	20%
3.	588/13	60/M	POORLY DIFFERENTIATED ADENOCARCINOMA	60%
4.	938/13	65/M	POORLY DIFFERENTIATED ADENOCARCINOMA	10%
5.	1397/13	55/F	MODERATELY DIFFERENTIATED ADENOCARCINOMA	30%
6.	2960/13	40/F	POORLY DIFFERENTIATED ADENOCARCINOMA	30%
7.	3118/13	75/M	WELL DIFFERENTIATED ADENOCARCINOMA	10%
8.	3227/13	50/F	MODERATELY DIFFERENTIATED ADENOCARCINOMA	60%
9.	3265/13	50/M	PAPILLARY ADENOCARCINOMA	15%
10.	3271/13	35/F	MODERATELY DIFFERENTIATED ADENOCARCINOMA	70%
11.	3281/13	70/F	SIGNET RING CELL CARCINOMA	30%
12.	3719/13	65/F	MODERATELY DIFFERENTIATED ADENOCARCINOMA	50%
13.	230/14	60/M	WELL DIFFERENTIAED ADENOCARCINOMA	15%
14.	298/14	60/M	WELL DIFFERENTIATED ADENOCARCINOMA	50%
15.	329/14	62/F	MODERATELY DIFFERENTIATED ADENOCARCINOMA	30%

16.	624/14	60/M	POORLY DIFFERENTIATED ADENOCARCINOMA	70%
17.	864/14	70/F	WELL DIFFERENTIATED ADENOCARCINOMA	60%
18.	974/14	60/M	WELL DIFFERENTIATED ADENOCARCINOMA	70%
19.	1002/14	44/F	POORLY DIFFERENTIATED ADENOCARCINOMA	20%
20.	1147/14	55/M	WELL DIFFERENTIATED ADENOCARCINOMA	40%
21.	1160/14	52/F	WELL DIFFERENTIATED ADENOCARCINOMA	10%
22.	1203/14	52/M	WELL DIFFERENTIATED ADENOCARCINOMA	20%
23.	1237/14	35/M	WELL DIFFERENTIATED ADENOCARCINOMA	20%
24.	1414/14	45/M	POORLY DIFFERENTIATED ADENOCARCINOMA	10%
25.	1735/14	45/F	MODERATELY DIFFERENTIATED ADENOCARCINOMA	50%

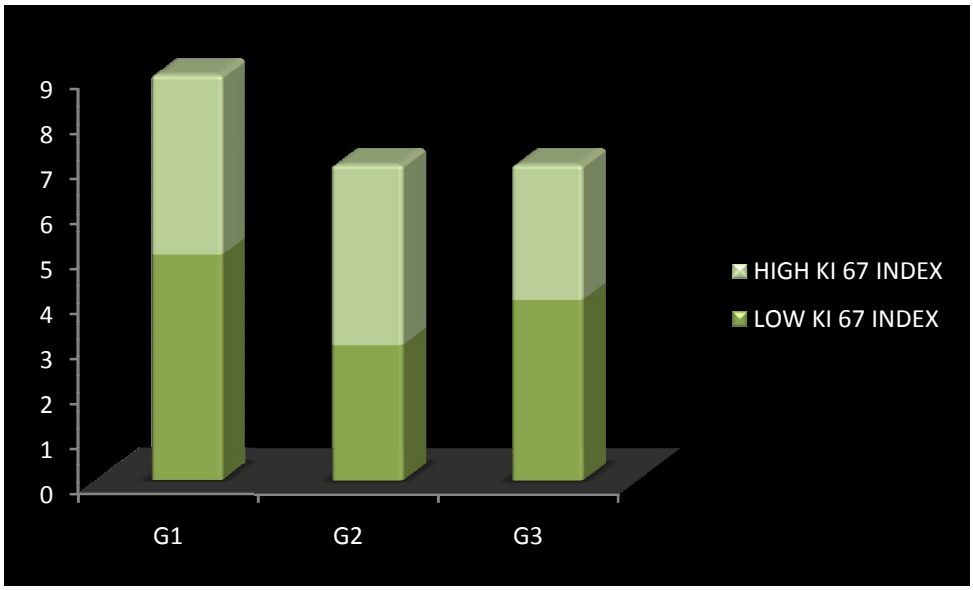
The Ki 67 labelling index was obtained for 25 cases including 23 cases of tubular adenocarcinoma, 1 case of papillary adenocarcinoma and 1 case of signet ring cell adenocarcinoma. The mean value was calculated to be 36.4%. Hence tumours with proliferation index <36.4%were categorized as low and >36.4% were categorized as high proliferative activity. **Chi square = 0.4. p> 0.05. Not significant. There is no association between the grade and Ki 67 expression.**

TABLE 15: KI 67 INDEX IN GASTRIC CARCINOMA

GRADE	LOW KI 67 INDEX	HIGH KI 67 INDEX
G1	5	4
G2	3	4
G3	4	3

In this study the number of tumours with a high proliferation index was 11, when compared to 12 cases with a low proliferation index. The average proliferation index for well differentiated tumours is 32.78%, for moderately differentiated tumours it is 44.28%, poorly differentiated tumours have an average proliferation index of 37.14%.

CHART 15: KI 67 IN GASTRIC CARCINOMA



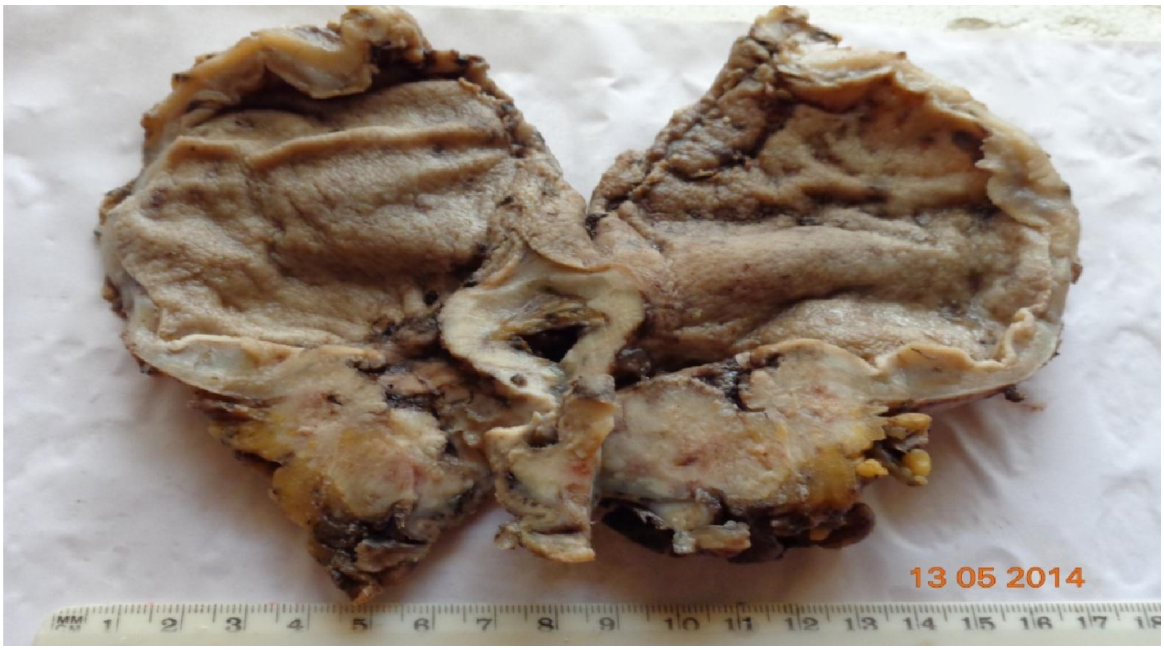


Figure 1: 1505/14- Gastrectomy specimen. Reveals an ulceroproliferative growth measuring 5x4 cm in the antrum. Adjacent mucosa ironed out. c/s of the growth grey white.

Figure 2: Partial gastrectomy specimen with an ulcerated growth measuring 4x3 cm located in the antrum. c/s- The growth grossly extends upto the serosa.

Figure 3:1414/14: Gastrectomy specimen showing superficially ulcerated plaque. It is accompanied by Conspicuous thickening of gastric wall. Adjacent mucosa ironed out.

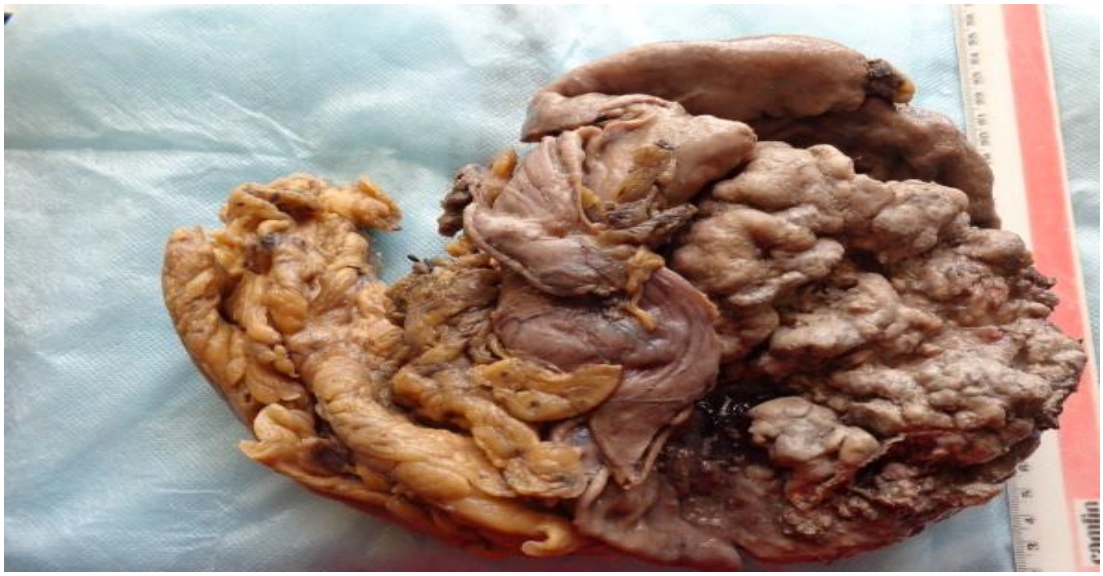


Figure 4 : 2315/12 Total Gastrectomy specimen with a growth measuring 15x 10 cm involving almost the entire stomach.The growth is nodular with focal areas of ulceration. A portion of large intestine was attached to the stomach.

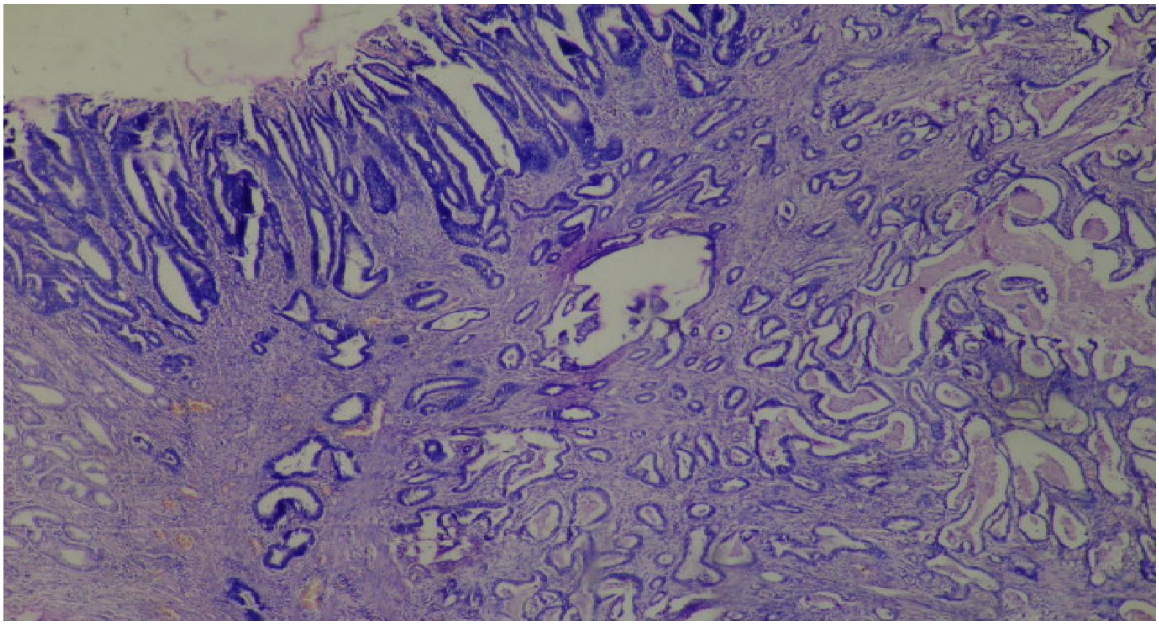


Figure 5: 230/14 – Well differentiated adenocarcinoma. H/E section shows a tumour composed of malignant cells in a predominantly glandular pattern invading the submucosa.(Scanner View)

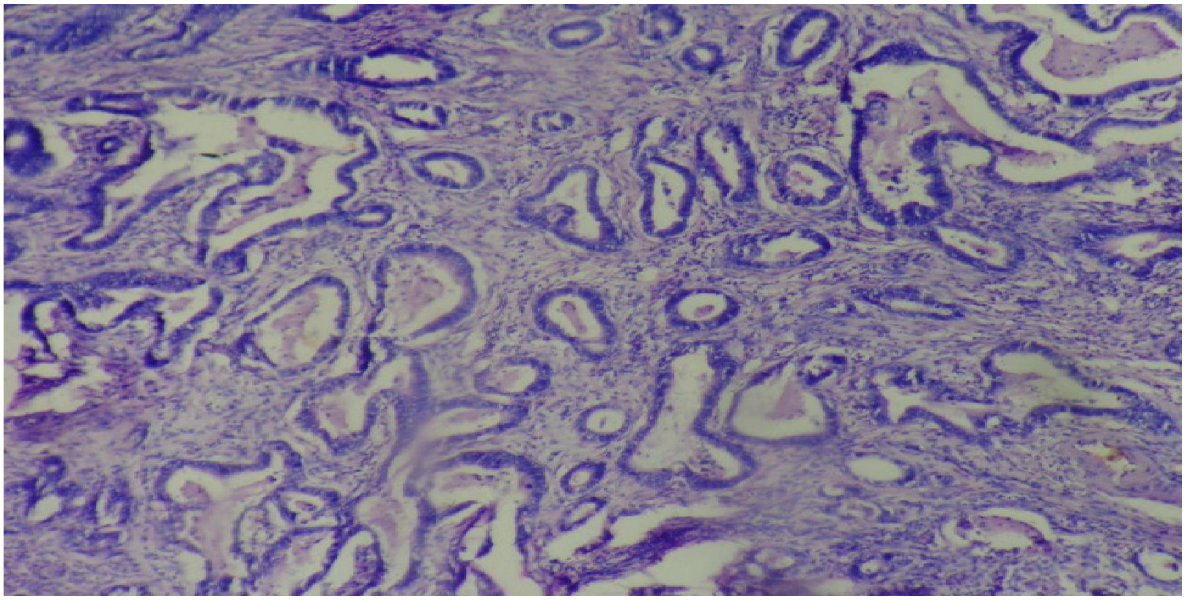


Figure 6: 230/14 - Tumour is composed of predominantly malignant glands in a tubular pattern showing irregular budding, branching and anastamosis.(10x)

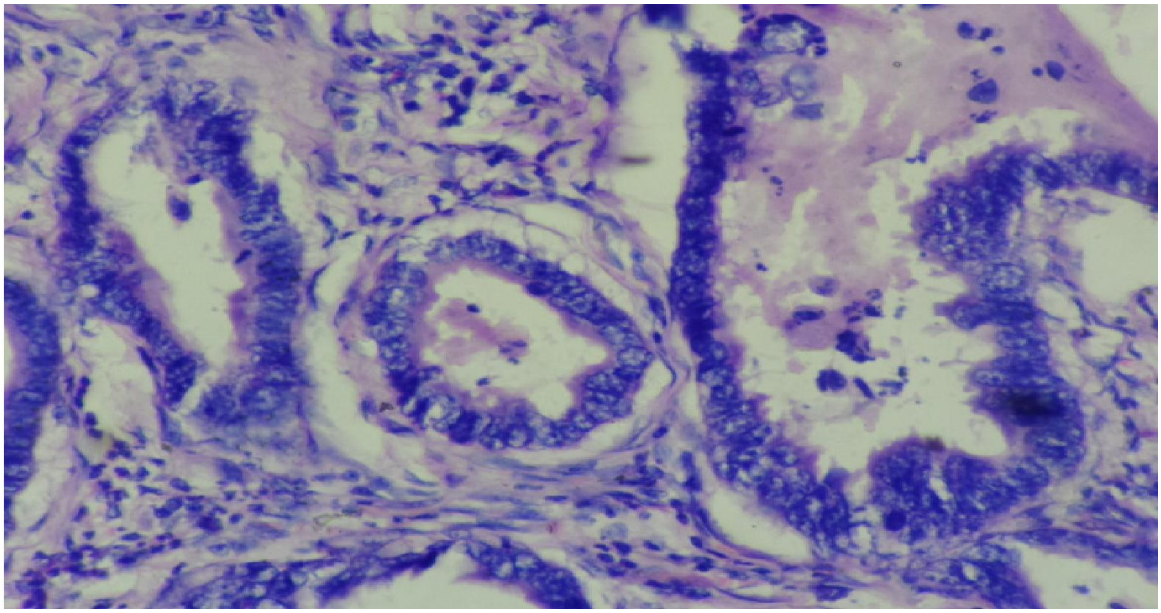


Figure 7: 230/14- Malignant glands are lined by cuboidal to columnar cells with a pseudostratified, pleomorphic vesicular nucleus with prominent nucleoli exhibiting loss of polarity. (40x)

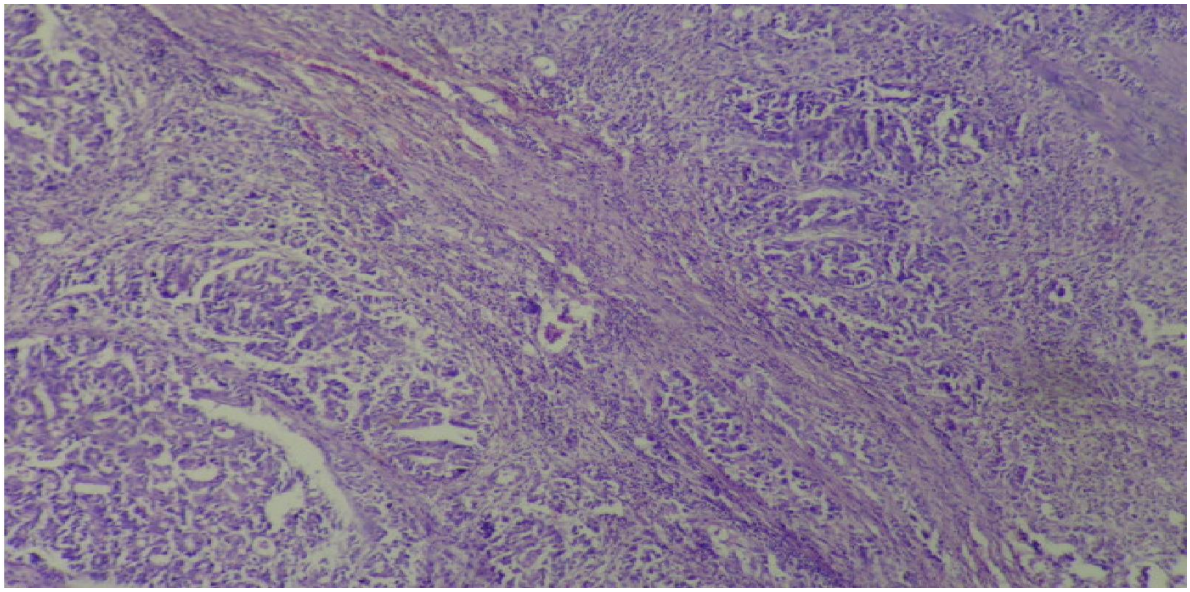


Figure 8: 3724/13 – Moderately differentiated adenocarcinoma. Tumour is composed of glandular component admixed with solid sheets of neoplatic cells. (10x)

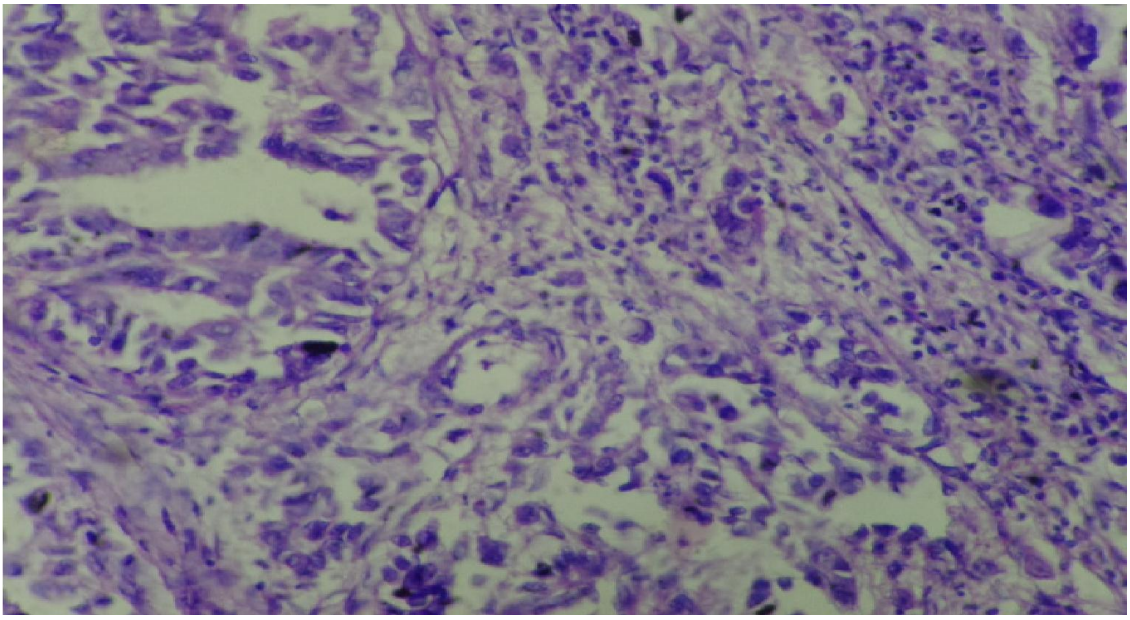


Figure 9: 3724/14- Moderate differentiated adenocarcinoma. The neoplastic glands are lined by cuboidal cells with basally situated pleomorphic vesicular nucleus with loss of cytoplasmic mucin

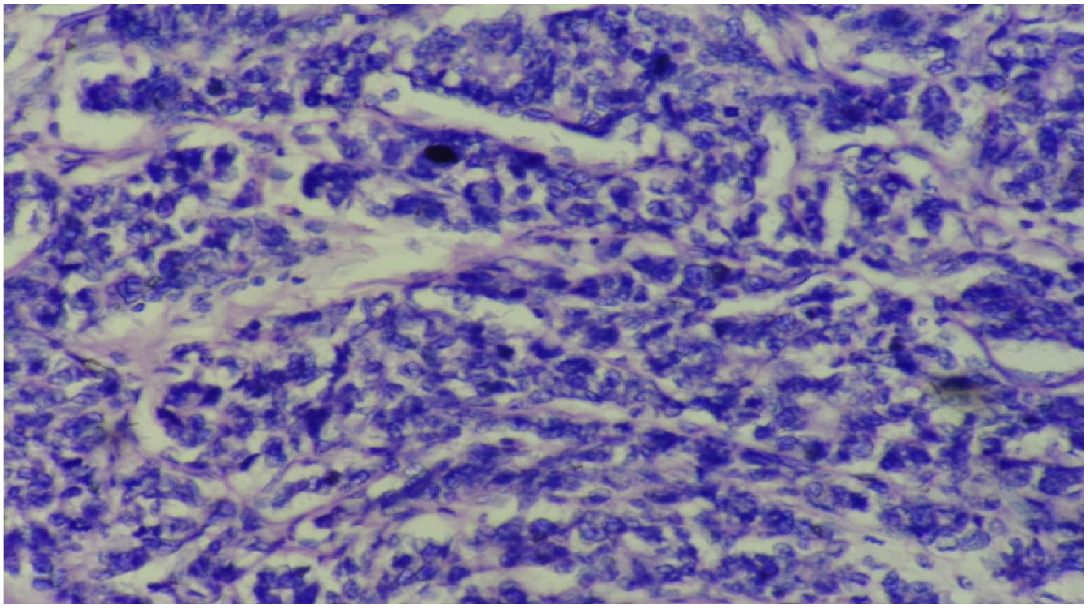


Figure 10: 624/14- Poorly differentiated adenocarcinoma. Composed of neoplastic cells arranged predominantly in a solid pattern with sparse glandular elements.(40x)

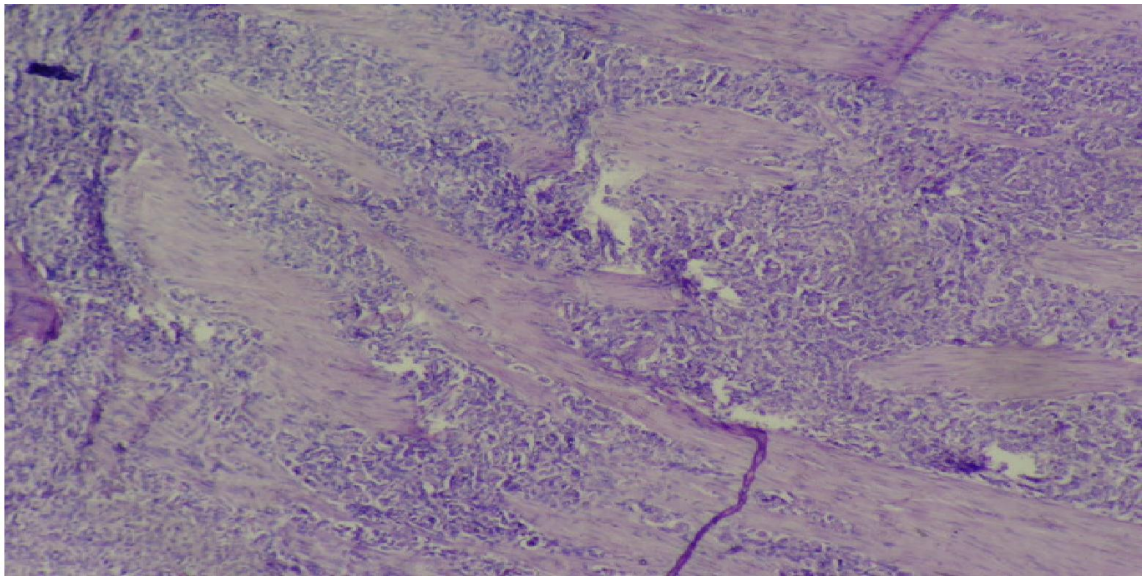


Figure 11: 624/14- Poorly differentiated adenocarcinoma. Tumour is composed of poorly cohesive cells predominantly in a dispersed pattern. Malignant cells are seen infiltrating the muscularis propria.(10x)

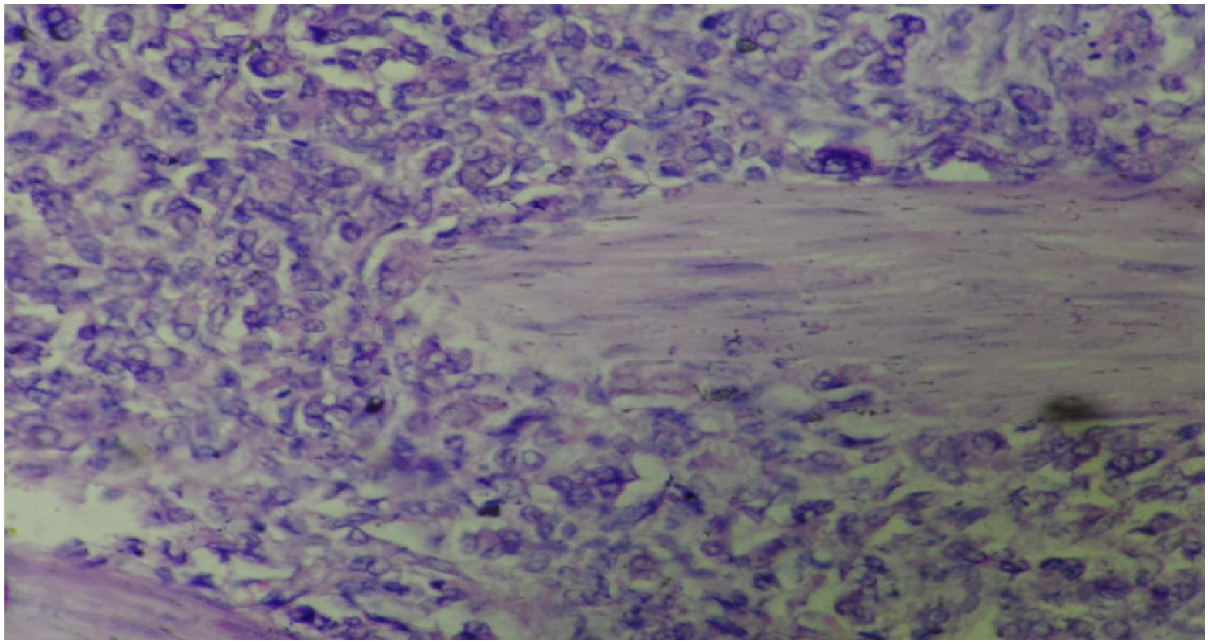


Figure 12:624/14- Poorly differentiated adenocarcinoma. Composed predominantly of sheets of dispersed cells having a vesicular pleomorphic nucleus infiltrating between the muscle bundles.(40x)

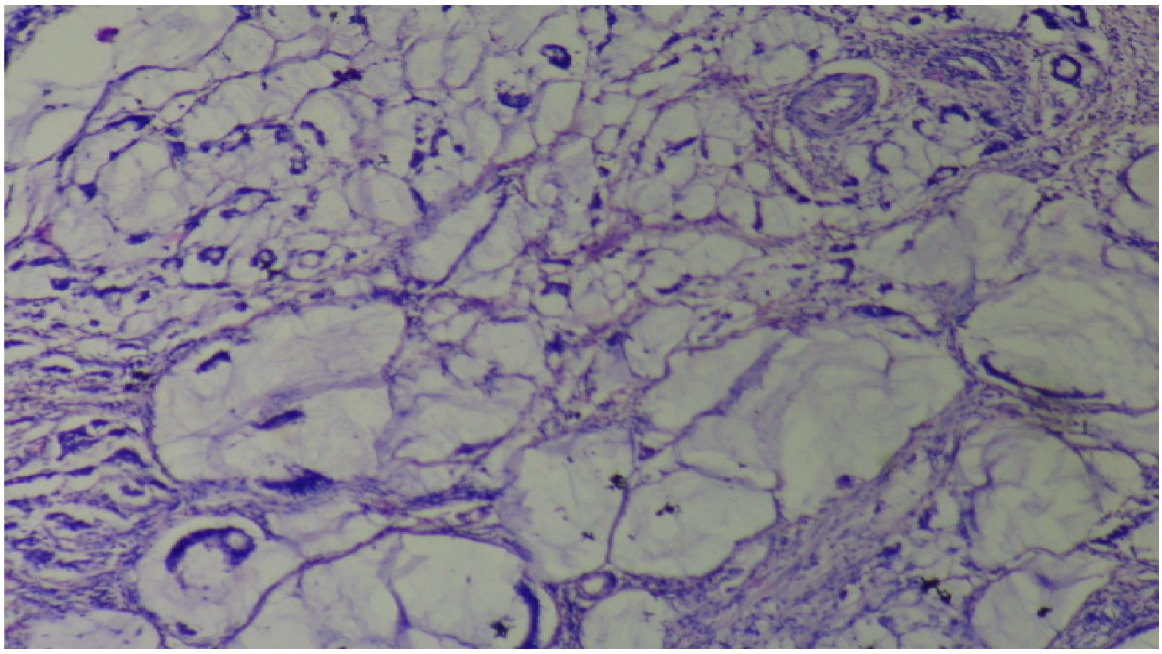


Figure 13: 814/14 – Mucinous adenocarcinoma. Tumour is characterized by copious amount of extracellular mucin forming >50% of the tumour volume.

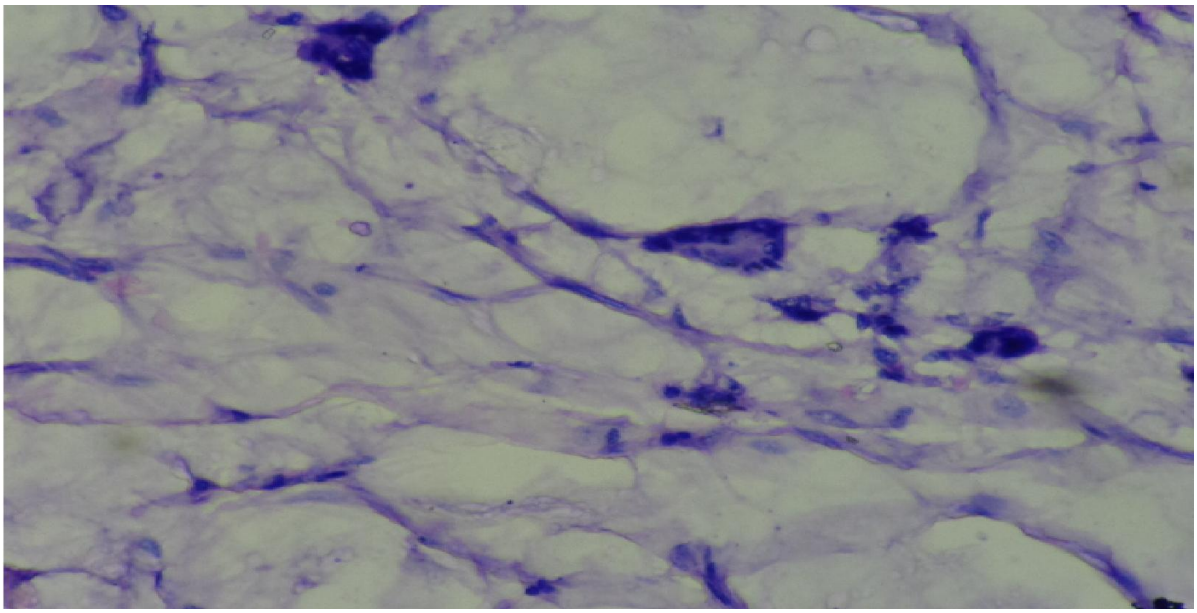


Figure 14: 814/14- Mucinous adenocarcinoma showing clusters of malignant cells exhibiting pleomorphism and hyperchromasia floating in pools of mucin. (40x)

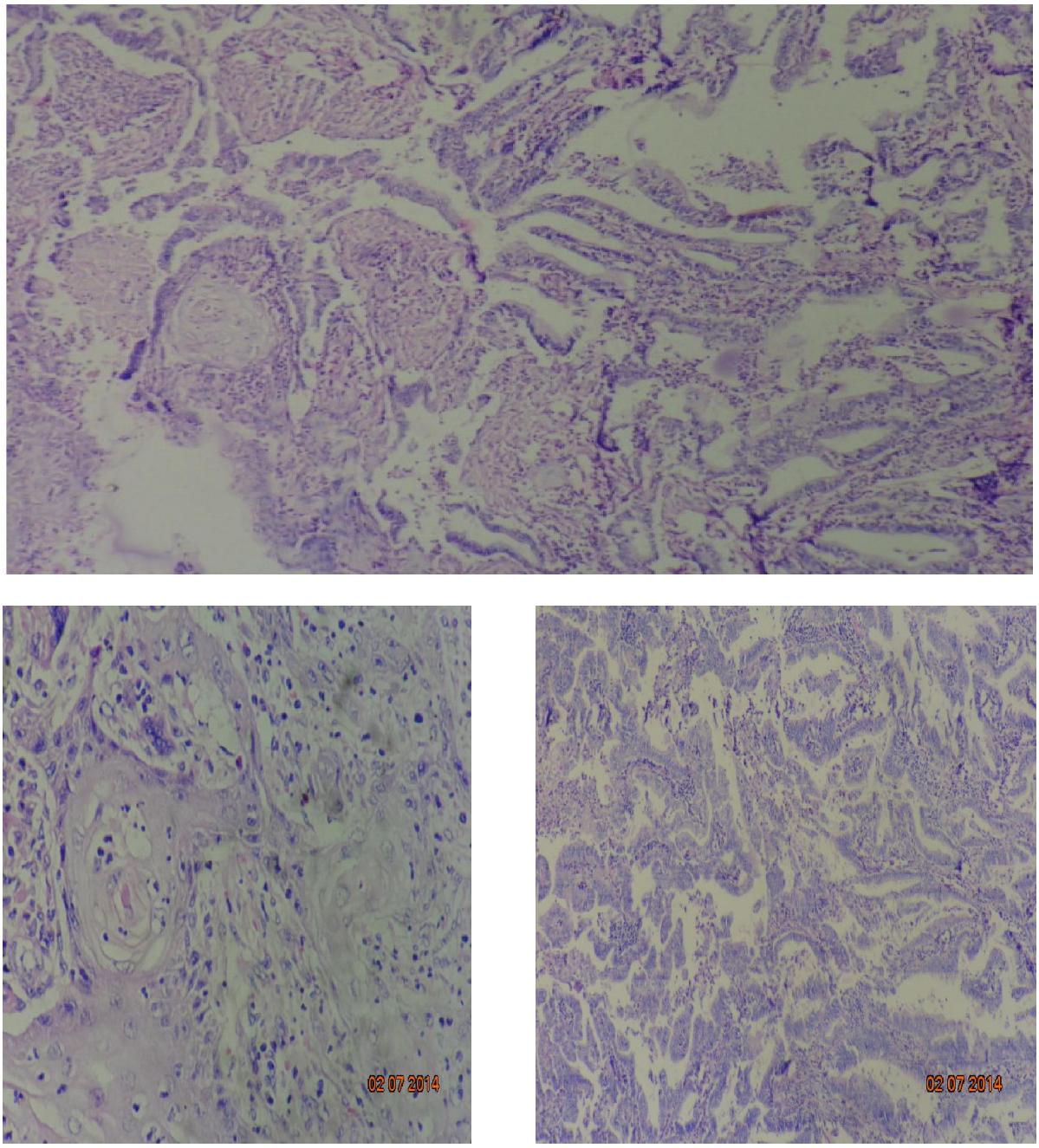


Figure 15, 16, 17. 1505/14- Adenosquamous Carcinoma. The tumour is composed of an admixture of varying proportion of adenocarcinoma and squamous cell carcinoma. The squamous component forms >25% of the tumour volume. The squamous component shows malignant keratin pearl formation.

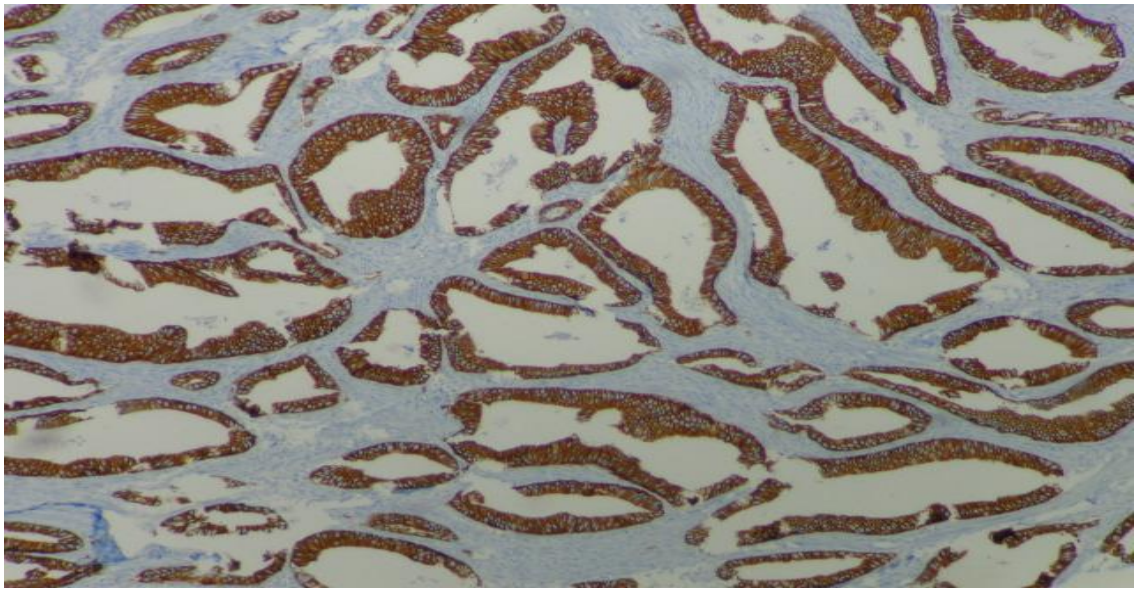


Figure 18: 1147/14- HER2 Score 3+ Well differentiated adenocarcinoma showing Intense staining with HER2.

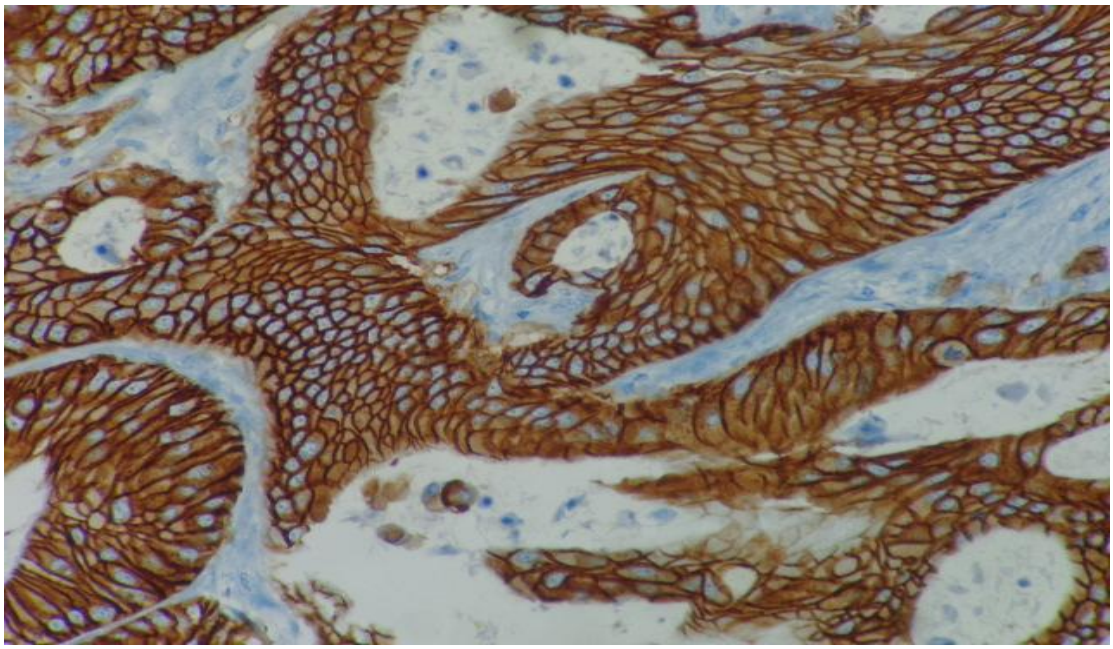


Figure 19: 1147/14-. HER2 score- 3+ Malignant glands showing complete and strong membrane positivity

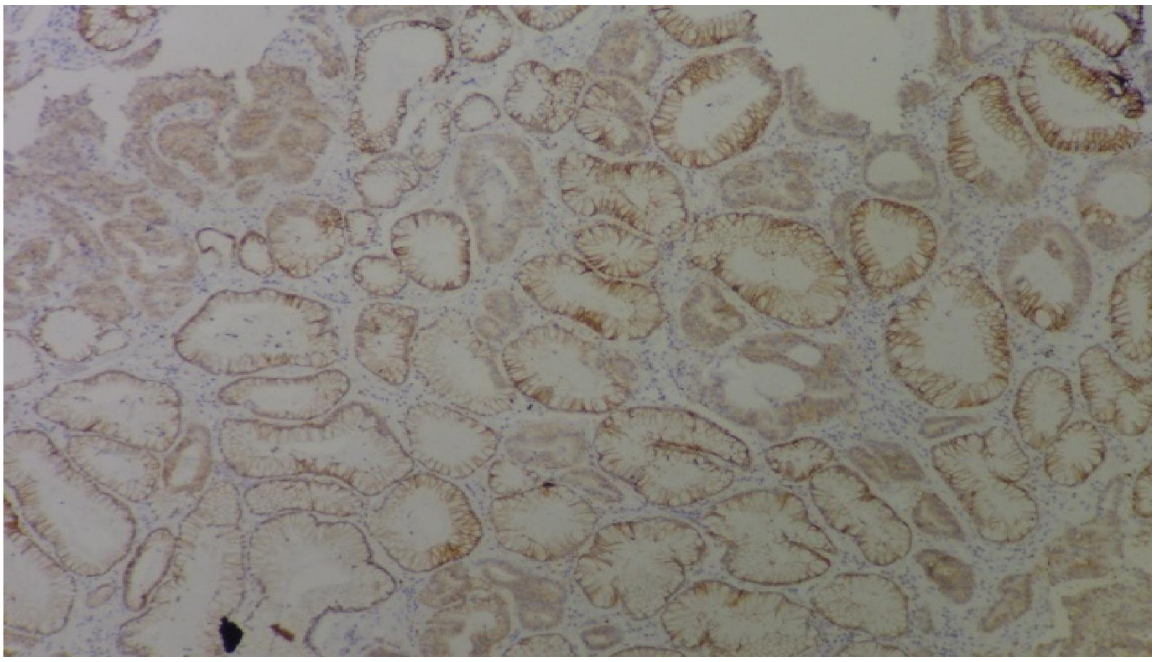


Figure 20: 3265/13- HER2 Score 3+: well differentiated adenocarcinoma showing 3+ membrane positivity in >10% of tumour cells.(10x)

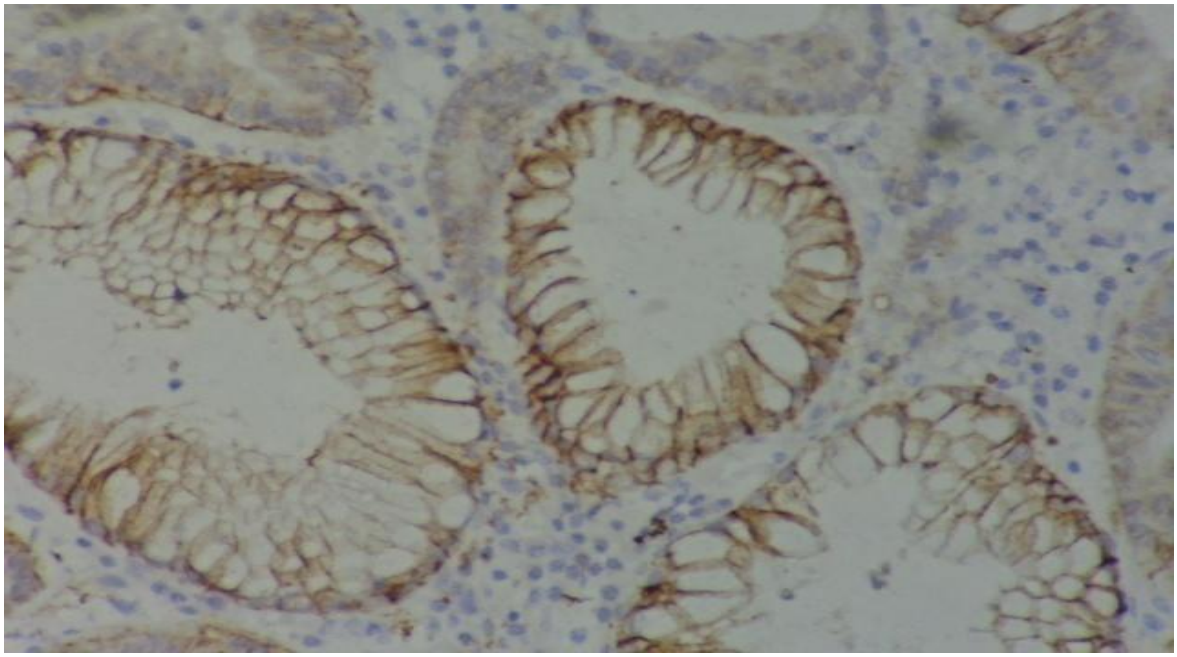


Figure 21: 3265/14 – HER2 score of 3+ : Malignant glands showing complete/Basolateral membrane positivity.

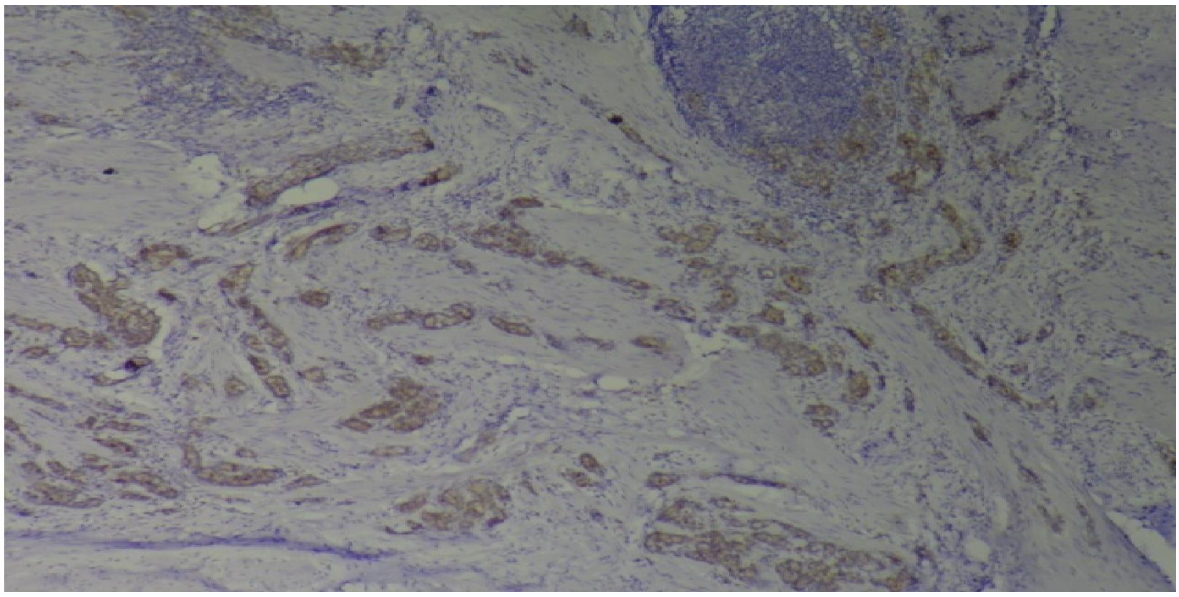


Figure 22: 1203/14 HER2 Score 2+ : Well differentiated adenocarcinoma showing HER2 score of 2+.(10x)

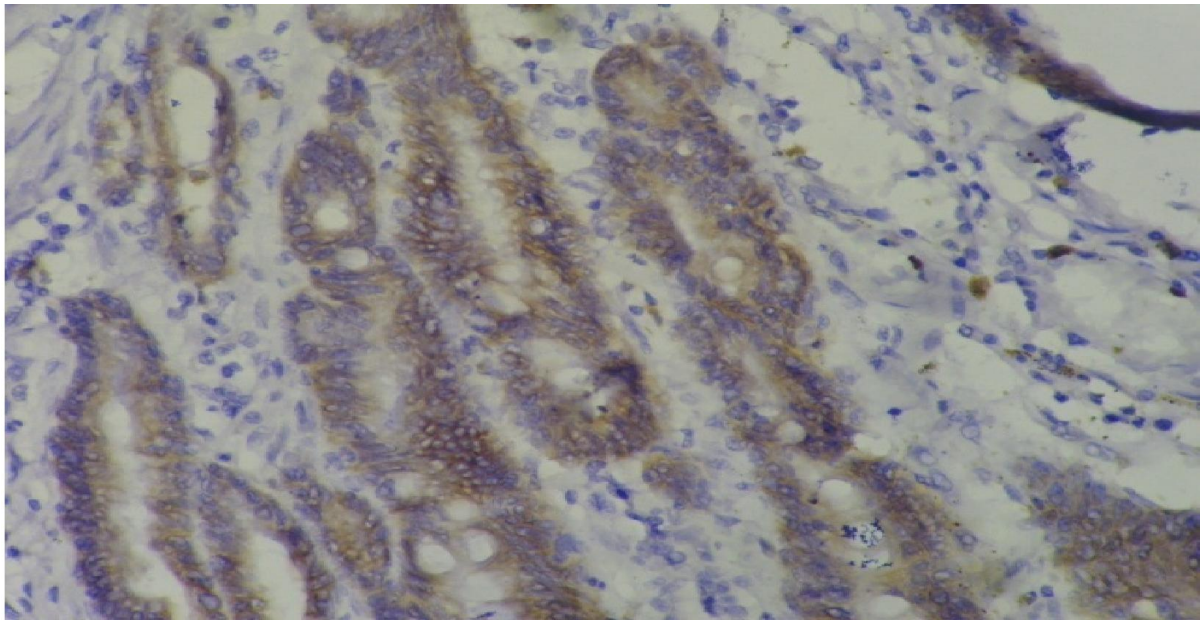


Figure 23: 1203/14- - HER2 Score 2+ : Weak to moderate complete or basolateral/ cytoplasmic membrane positivity in 10% of tumour cells. (40x)

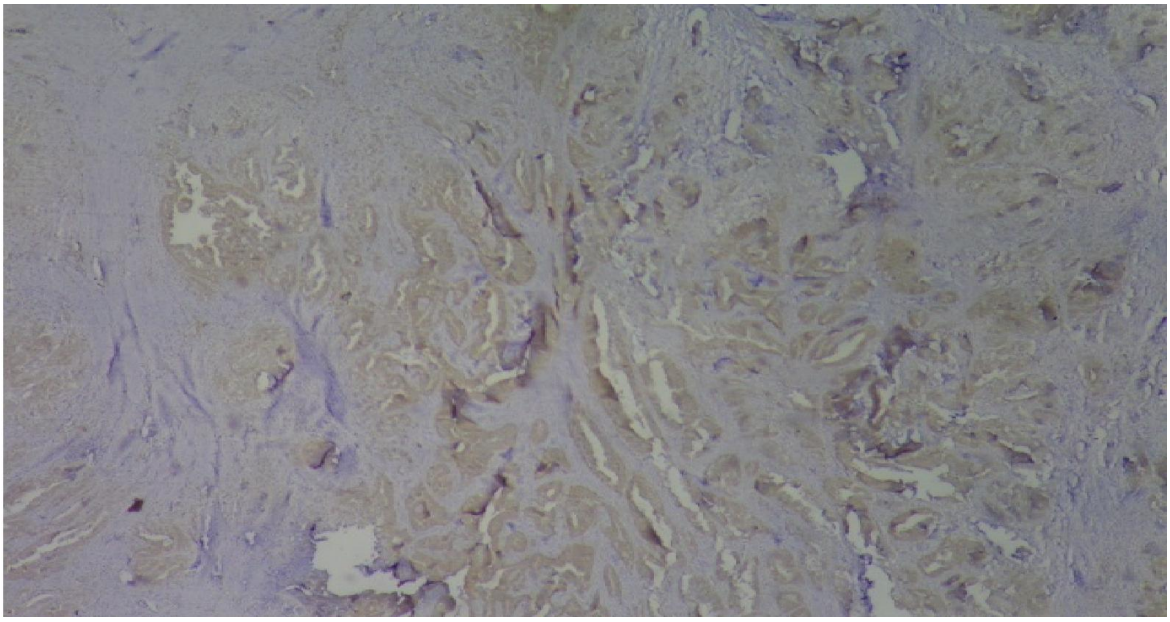


Figure 24: 1237/14 : HER2 score 1+ : well differentiated adenocarcinoma showing IHC score of 1+.(10x)

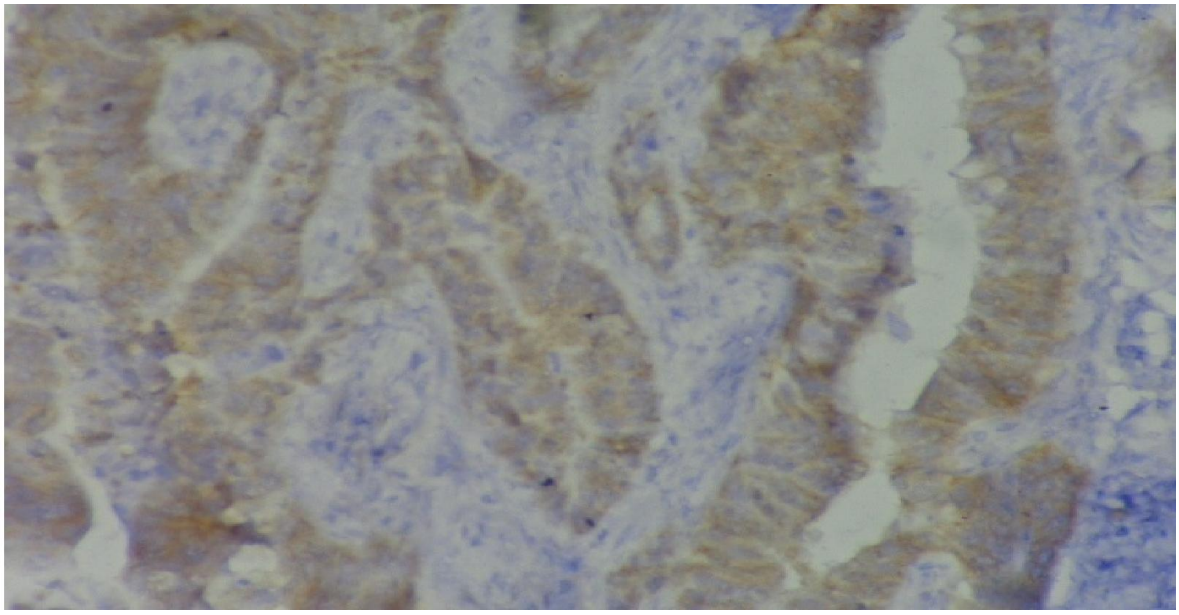


Figure 25: 1237/14- HER2 Score 1+ : Faint cytoplasmic membrane positivity in 10% of the cells.

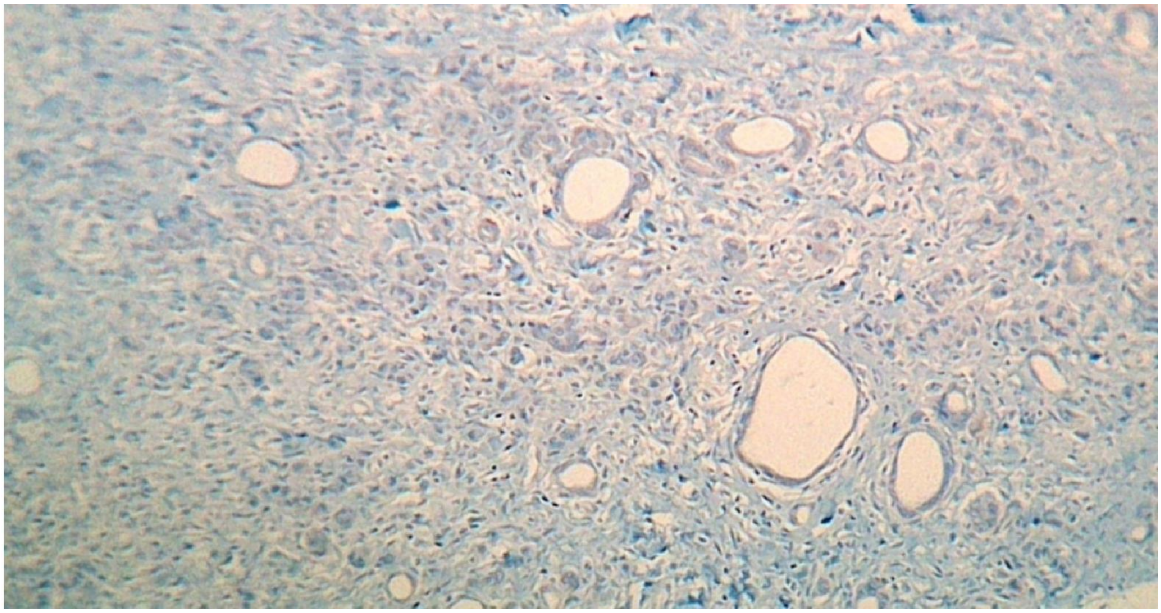


Figure 26 : 2315/13 – HER2 Score 0- No membranous reactivity in the Malignant epithelial cells.

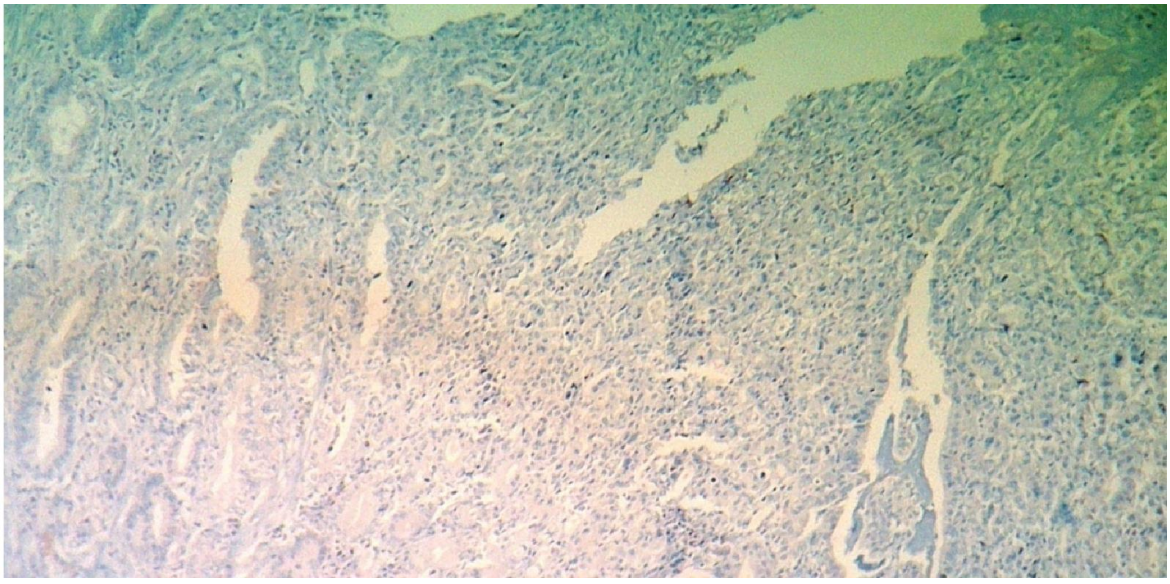


Figure 27 : 230/14 – HER2 score 0 – No membranous reactivity in the tumour cells.(40x)

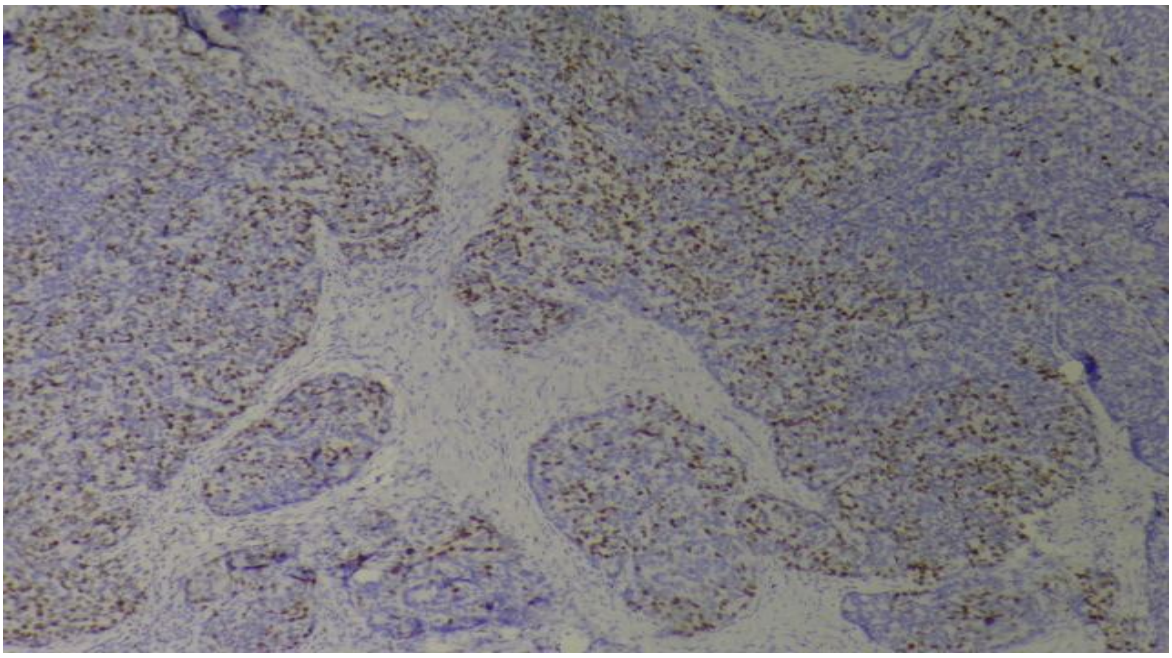


Figure 28 : 588/13 – Poorly differentiated adenocarcinoma showing high Ki 67 expression.(10x)

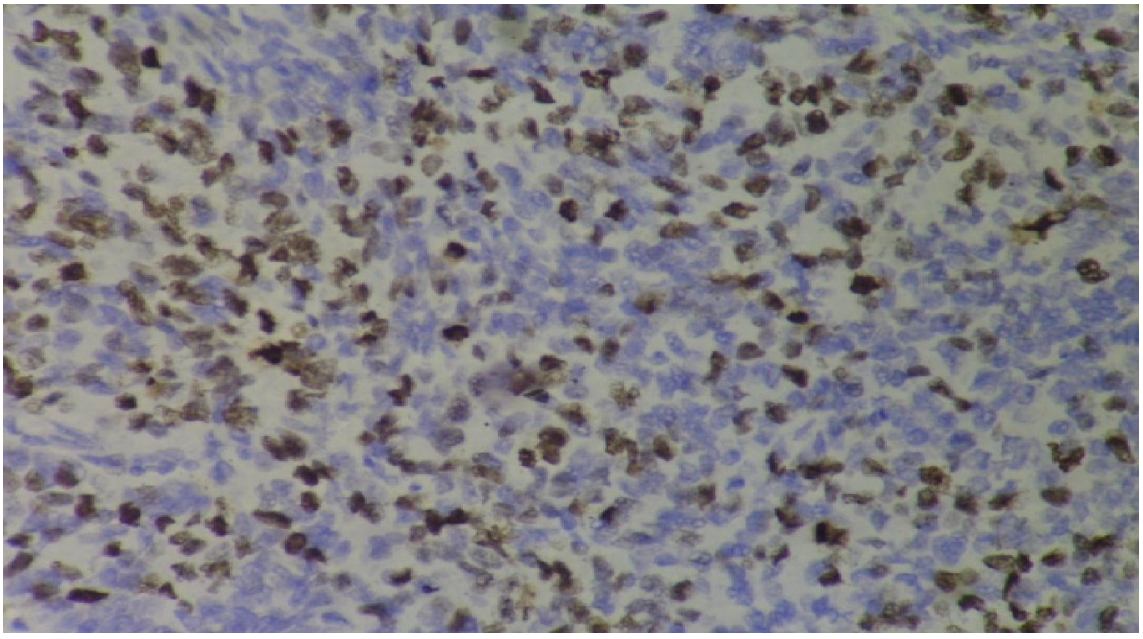


Figure 29: 588/13- Malignant epithelial cells showing strong nuclear positivity of Ki 67.

(40x)

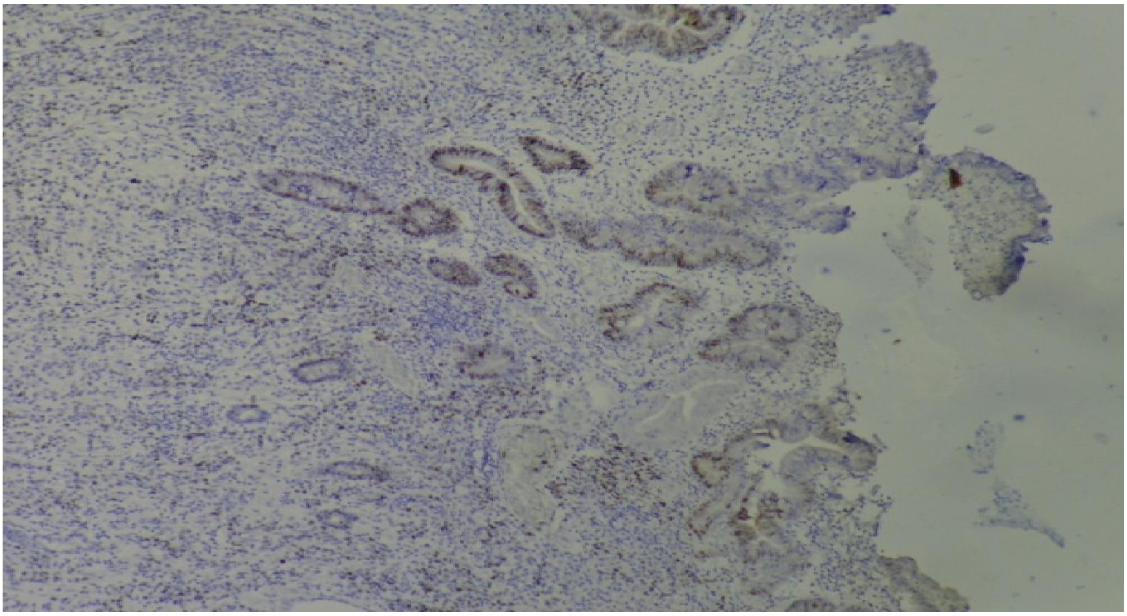


Figure 30: 1203/14 – Well differentiated adenocarcinoma with malignant glandular elements showing low Ki 67 expression.(10x)

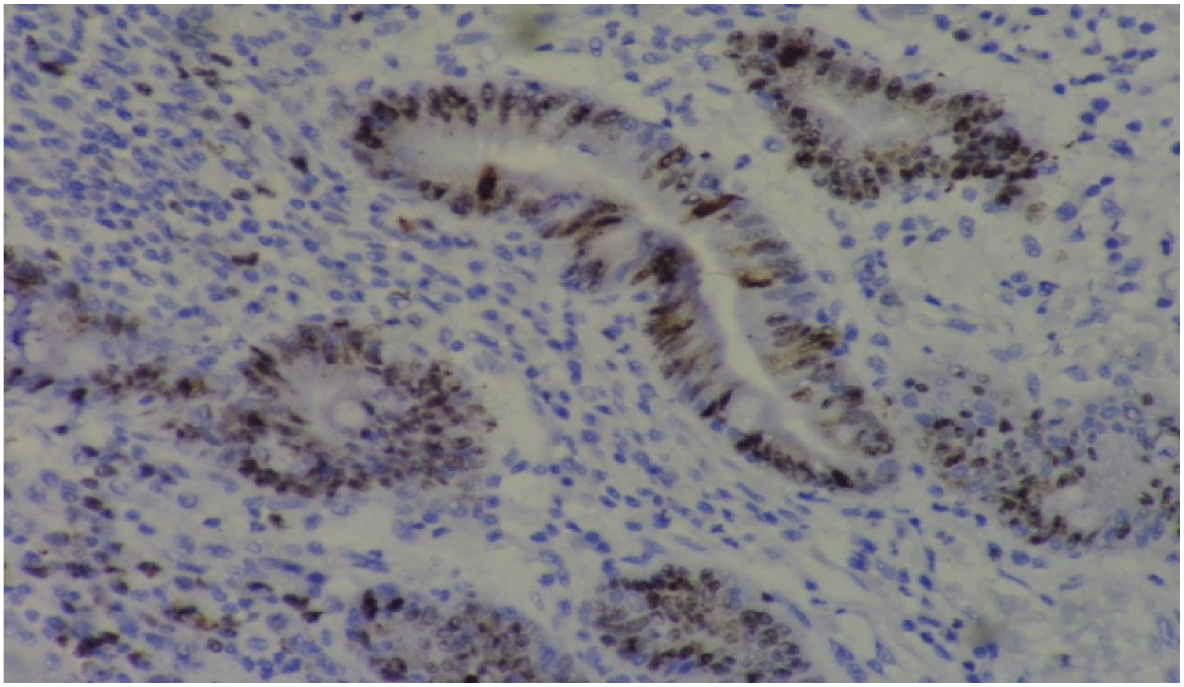


Figure 31: 1203/14- Nucleus exhibiting Ki 67 positive cells lining the glandular epithelium.

(40x).

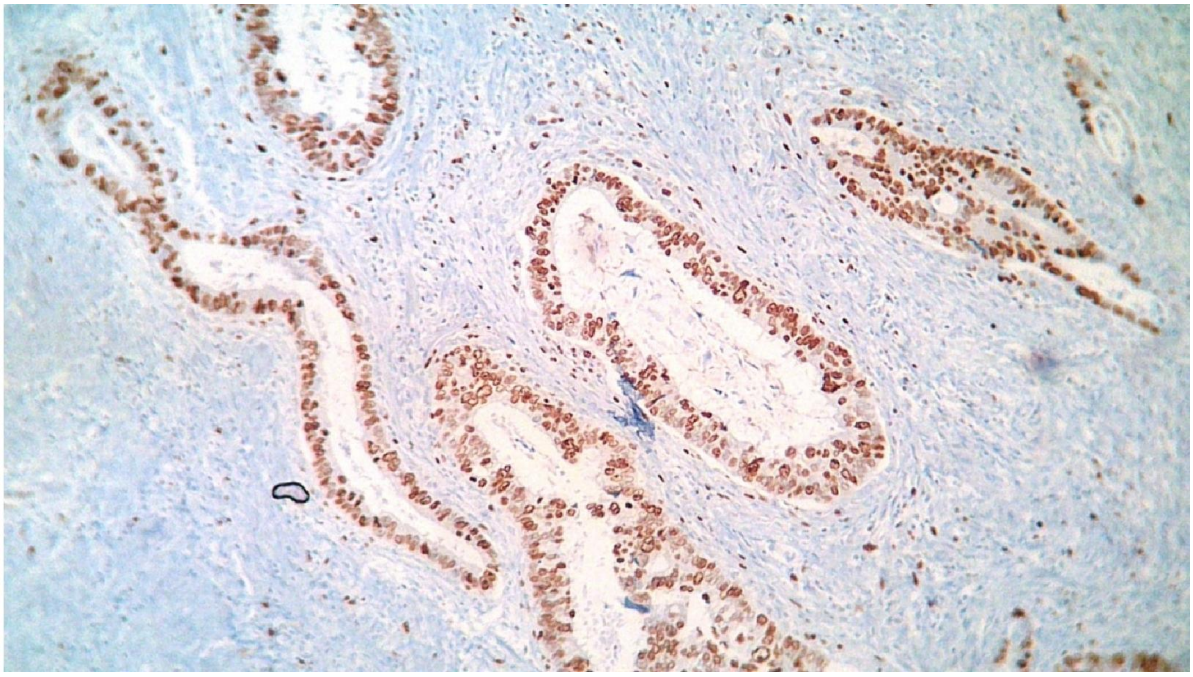


Figure 32: 974/14 : Well differentiated adenocarcinoma. Nucleus of malignant glandular epithelium showing strong nuclear positivity of Ki 67.(40x)

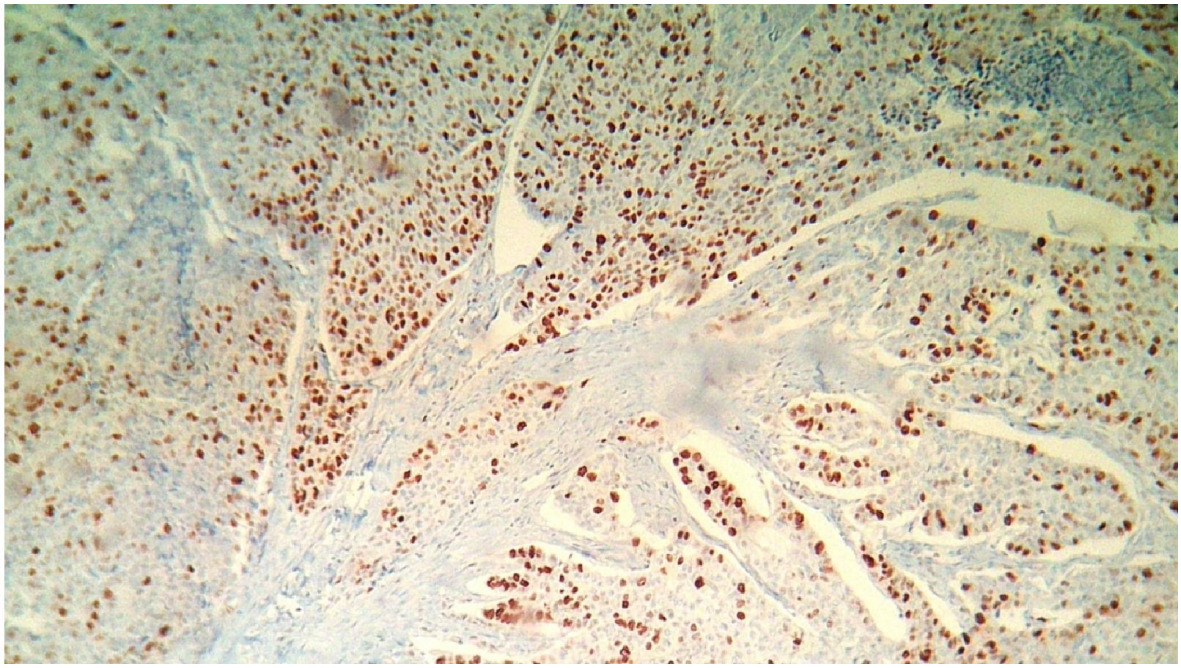


Figure 33: 1203/14- Well differentiated adenocarcinoma showing strong nuclear positivity.(10x)

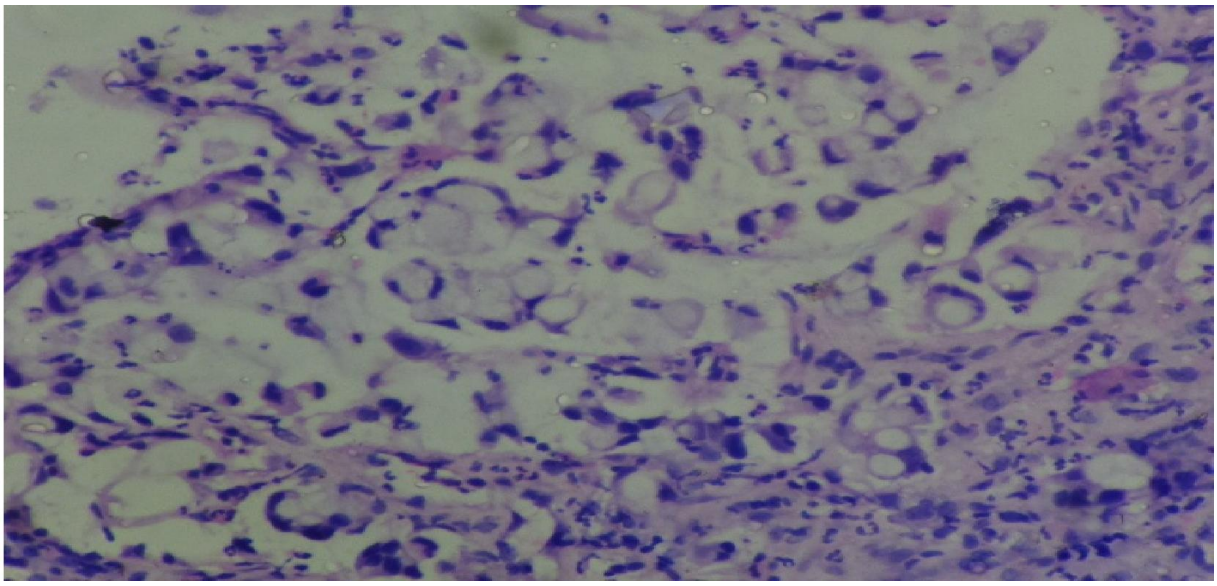


Figure 34: 3281/13 : Signet Ring cell carcinoma Composed predominantly of single cells or isolated cells. The cells have a prominent intracytoplasmic mucin vacuole and an eccentrically placed hyperchromatic nucleus.(40x)

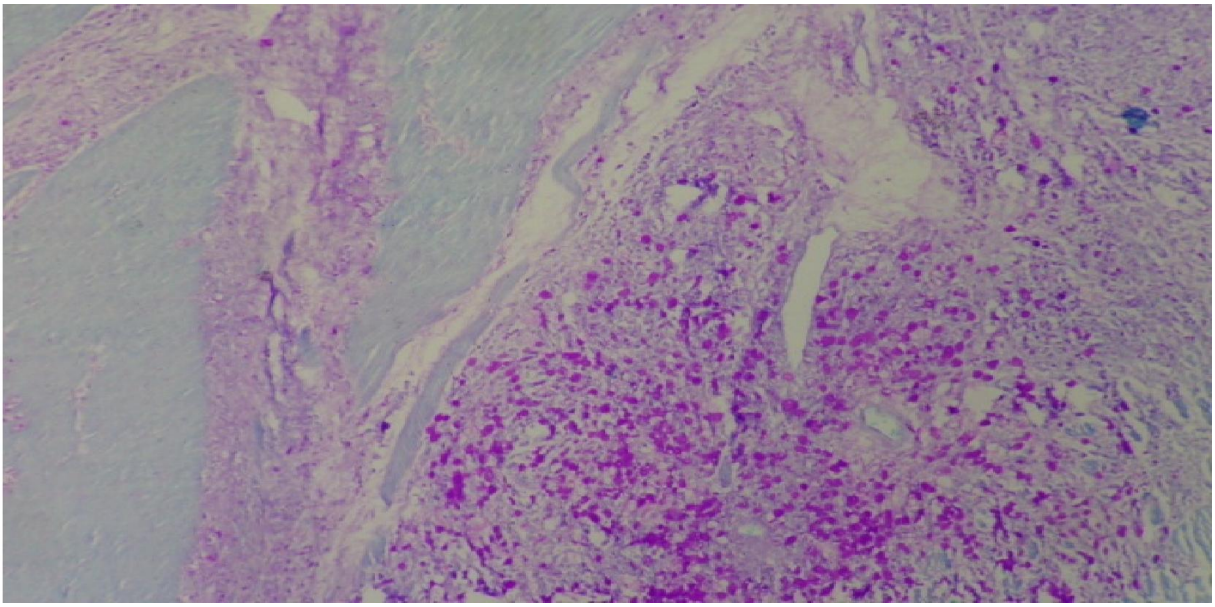
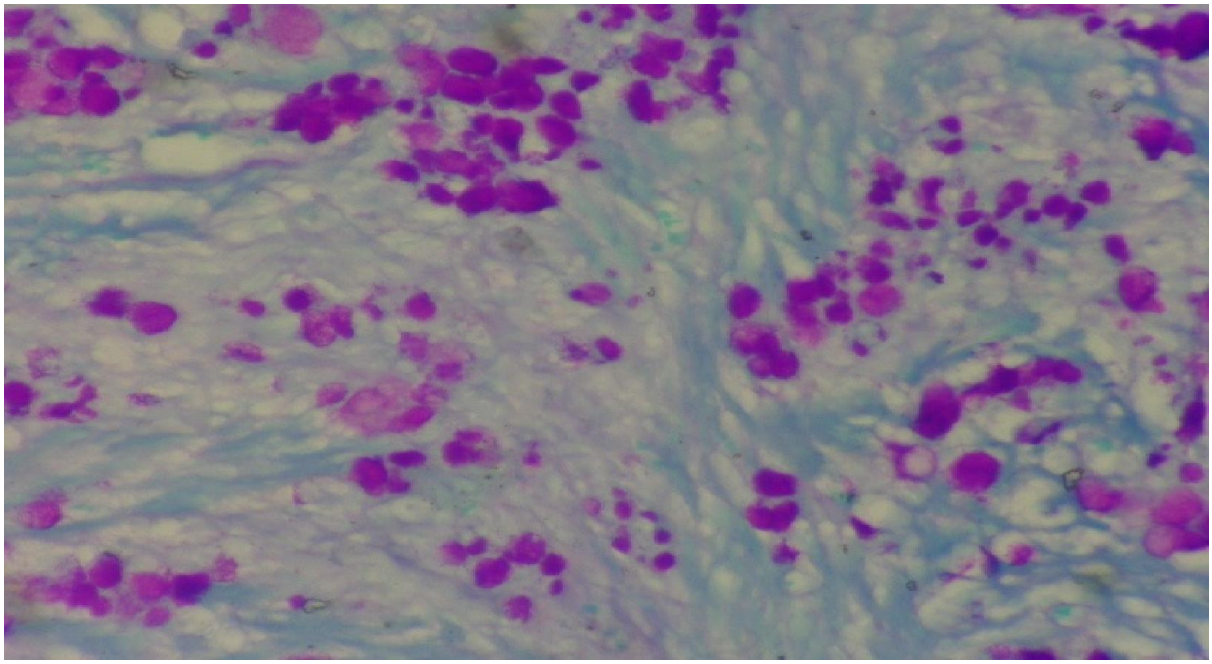


Figure 35 : 3281/ 13 Signet Ring cell carcinoma Stained with Periodic Acid Schiff. The tumour is composed of individual cells separated by a fibrous stroma (10x)



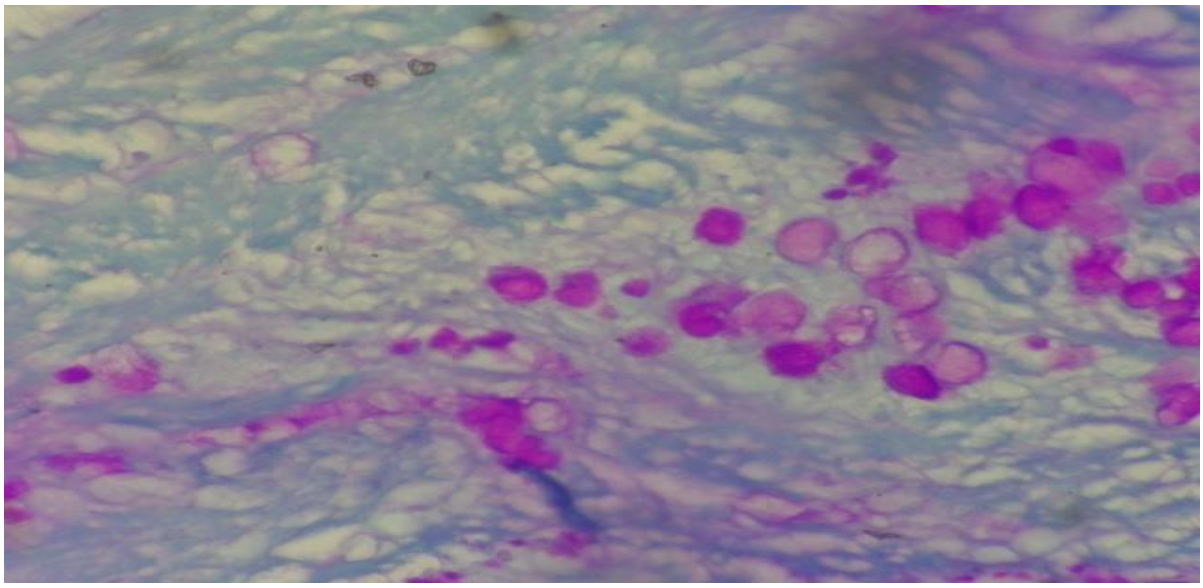


Figure 36 & 37: 3069/13 – Signet Ring cell carcinoma. (40x)- Periodic acid Schiff demonstrates intracytoplasmic mucin vacuole that has taken up the magenta stain.(40x)

DISCUSSION

DISCUSSION

Gastric carcinoma ranks among the top five cancers of the world. Its the fourth most common cancer in terms of incidence. It is the second biggest killer among all cancers.

According to Inoue et al[40] stomach cancer stands fourth among the major killers. It stands next to cancer of lung, breast and colorectum with its incidence of 9% . It closely follows lung cancer as the second most important killer (10%).

It has been estimated that by the year 2020 the world population would exceed 75 million. Among this nearly 15 million individuals would be affected by cancer. There will be a cancer related mortality of 12 million[40]. A phenomenon referred to as "The unplanned triumph" has emerged in the field of gastric cancer. It is the widespread decrease in incidence in the last 50 years. Inspite of all this cancer of stomach continues to rank among the top five important killers.

According to a study in Australia, known for its low incidence and prevalence, gastric carcinoma the disease remains to be the sixth leading cause of death. A study carried out in USA has reported an incidence of 7.3/100000 in males and 3.6/100000 in case of its female population[38].

Japan was once considered as the world leader in gastric carcinoma. It showed a change in trend with the lung cancer being the prime killer in men and colorectal cancer in women[3]. Katherine et al[41] reported two third of the gastric carcinomas occurred in the developing countries of the world. Countries like Japan and Korea form the biggest contributers.

According to NCIN data base people of Indian ethnicity have lowered rates of cancer incidence when compared to the natives of England[42]. India known for its diversity in culture. Diet and ethnicity also play a role with a diverse presentation in its cancer statistics. According to the reports submitted by the 10th International gastric cancer congress, incidence of gastric

carcinoma among Indian male is 11.8/100000 and among Indian females it is 5.9/100000. The mortality rates in male is 3.9/100000 and in females it is 2.1/100000[43].

Malhotra et al states that South Indian regions ranks first in the incidence of gastric carcinoma. It has the highest incidence when compared to the other regions of India[44].

The state of Mizoram was not included in the in the National Cancer Registry Programme till 2003. According to recent statistics it is said to have the highest prevalence in India[45].

A study by Imran Ali et al[35] states that maximum mortality in India is attributed to cancer. It causes nearly 0.3 million deaths every year, next to cardiovascular disorders. The annual prevalence was estimated to be around 2.5 million cases with the number of newly diagnosed cases being around 8,00,000. The number of cancer deaths being around 50,000 per year. Prevalence of gastric carcinoma is high in Mizoram and regions of north east India. Gastric carcinoma is the second most commonly reported malignancy in the states of Tamil Nadu, Andhra Pradesh, Sikkim and Goa. It is the third most common cancer in the state of Jammu and Kashmir.

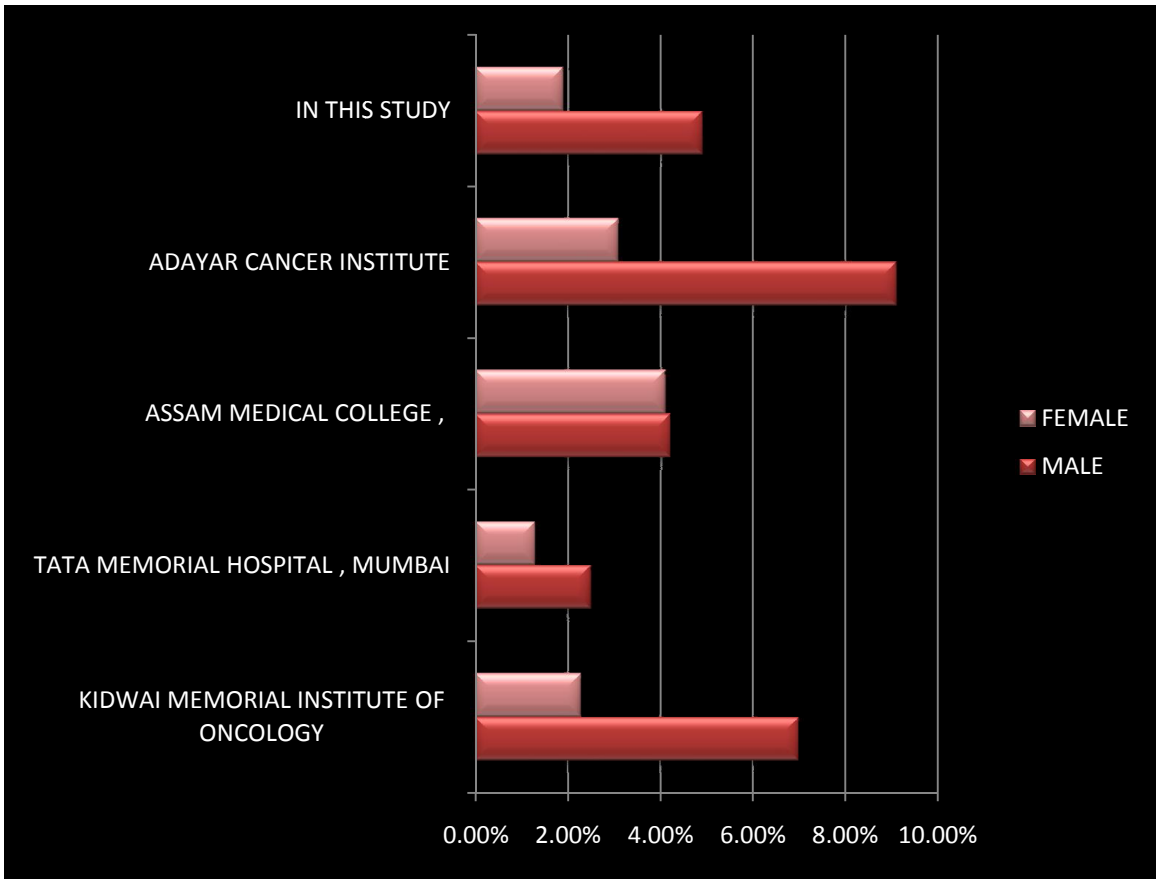
The incidence of Gastric carcinoma in our study carried out in Thanjavur medical college is 6.78% .It assumes fourth position following carcinomas of head and neck region, colorectum and breast. It is consistent with most of the world statistical reports. The incidence of Gastric carcinoma in AIIMS, New Delhi is 2.8% and it assumed the 11th position[46].

TABLE 16: INCIDENCE OF GASTRIC CARCINOMAS IN VARIOUS INSTITUTIONS ACROSS INDIA[47,48]

S. No	NAME OF THE INSTITUTE	MALE	FEMALE
1.	KIDWAI MEMORIAL INSTITUTE OF ONCOLOGY ,BANGALORE	7.0%	2.3%
2.	TATA MEMORIAL HOSPITAL , MUMBAI	2.5%	1.3%
3.	ASSAM MEDICAL COLLEGE , DIBRUGARH	4.2%	4.1%
4.	ADAYAR CANCER INSTITUTE, CHENNAI	9.1%	3.1%
5.	IN THIS STUDY	4.9%	1.9%

In this study the percentage of incidence of gastric carcinoma among all cancers in our institute is 4.9% in males and 1.9% in females. This is compared with the data from the major tertiary care centres across India . The Adayar cancer institute Chennai reports an incidence of 9.1% of cases in males and 3.1% cases in females. The Tata memorial hospital of Mumbai reports 2.5% of cases in males and 1.33% of cases in females. Kidwai institute of oncology Banglore show a statistics of 7% in males and 2.3% in females. Assam medical college has an incidence of 4.2% in males and 4.1% in females.

CHART 16 : INCIDENCE RATE OF GASTRIC CARCINOMA IN VARIOUS INSTITUTES ACROSS INDIA



According to the AIIMS statistics gastric carcinoma is the ninth leading cause of cancer death in males (3.5%) and the assumes the 13th position in cancer deaths among females (1.9%)[46]

The data given by the Mumbai cancer registry states that the annual age adjusted incidence rate for gastric carcinoma among men was 4% with about 1/ 337 individuals having the chance of developing cancer in his lifespan[49]. The annual incidence rate in men is 4.2% and in women is 2.2% with a mortality rate of 2.5% in male and 1.9% in female.

Roder et al[38] states that the male female ratio in gastric carcinoma was 1.8:1. This was reported in a study carried out in Centre for Cancer control and Research, Australia.

Most of the studies carried out in gastric carcinomas usually concentrate in urban & semi urban statistics. A study was carried out by Rajaraman et al in the Dindugal Ambilikai cancer centre which is a rural based population registry. Here the reported incidence in the male population was 5.6% and the female population was 2.5%. This is low when compared to the statistics from Chennai in the same year which was 11.1% for males and 5.3% for females.[51] Inspite of decreased incidence of this population had a decreased survival when compared with urban population. This is attributed to the lack of early screening and diagnostic facilities, availability of interventional practices and compliance of the patients to the treatment regimens.

AGE WISE INCIDENCE OF GASTRIC CARCINOMA

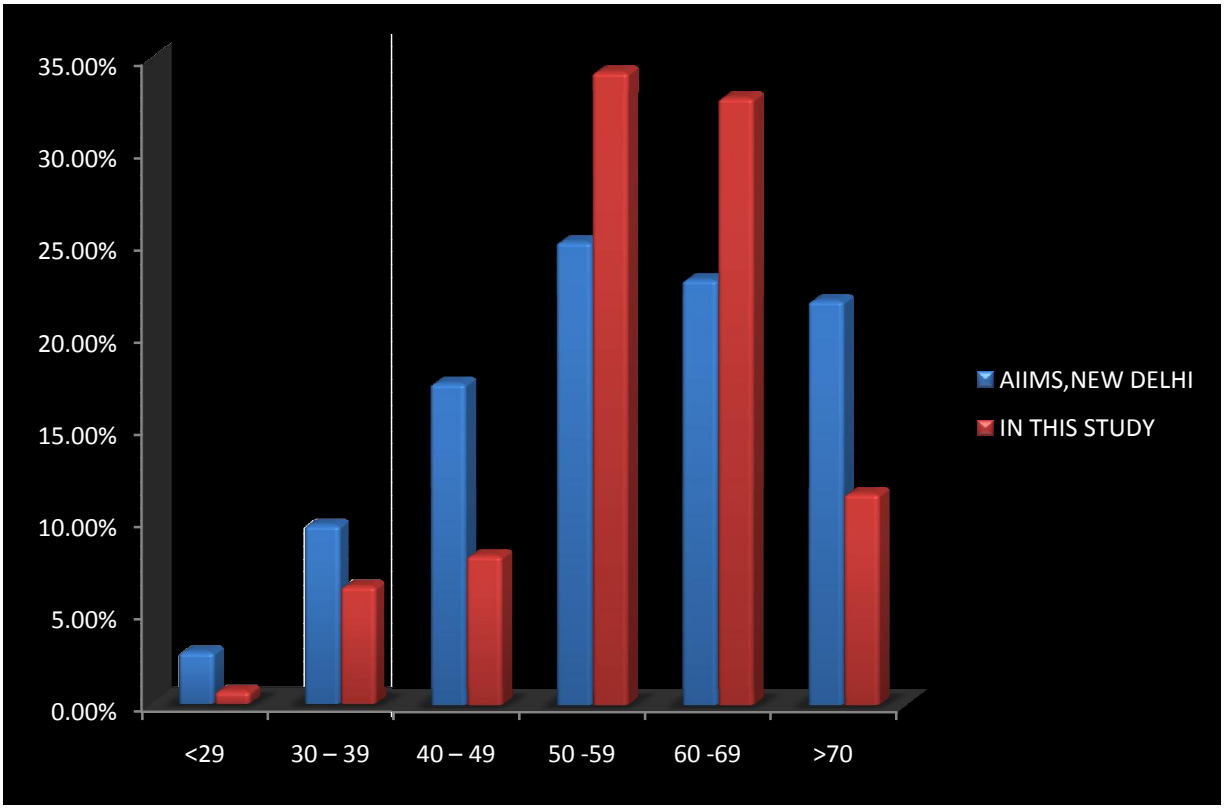
In our study the prevalences of gastric carcinoma in various age groups were analysed. It was found to be highest in the fifth to sixth decade of life (34.28%). This is closely followed by a prevalence of 32.28% in individuals in the age group of 60 - 69. The incidence is least in individuals under the age of 30 yrs. Our data correlates with the world statistics and the statistics of the leading tertiary care Institutes of India.

TABLE 17 : AGE WISE COMPARISON OF GASTRIC CARCINOMA AMONG VARIOUS TERTIARY CARE CENTRES

AGE IN YEARS	AIIMS,NEW DELHI		THANJAVUR MEDICAL COLLEGE	
	NO. OF CASES	% OF CASES	NO. OF CASES	% OF CASES
<29	7	2.83%	1	0.7%
30 – 39	24	9.71%	9	6.42%
40 – 49	43	17.40%	20	8.07%
50 -59	62	25.10%	48	34.28%
60 -69	57	23.07%	46	32.85%
>70	54	21.86%	16	11.42%

The statistics regarding the predominant age of incidence co related with the statistics of AIIMS, New Delhi. There the predominant age group affected was people in their fifth & sixth decade of life with 25.10% & 23.07% respectively. All studies showed the lowest incidence rate in individuals less than the age group of 29 years.

CHART 17: AGE WISE COMPARISON OF GASTRIC CARCINOMA AMONG VARIOUS TERTIARY CARE CENTRES



Katherine et al[41] states that the risk of Gastric carcinoma increases with age. It peaks during the age group of 50 – 70 years.

According to Inoue et al[40] ,the global trend of decrease in gastric cancer incidence is reflected in all age groups. The number of cases among younger individuals is on a decline.[40] His study compares the predominant age of occurrence during a span of time. In 1950 it was 61 years, where as in 2000 the common age of occurrence was around 70 years.

Malhotra et al[44] compares regional differences and age group of individuals with gastric cancer in India. According to him the onset of gastric cancer in people of south India starts around 35 -55 years. This is a decade earlier when compared to the age of 45 -55 years in the people of Northern territory. This was postulated to have occurred by the influence of the local diet on carcinogenesis.

Sipponen et al[52] compares the age of incidence with the sex. According to the study carried out in a Finnish Cancer Registry, the female population presented with a delay of around 10 -15 years when compared to males. This was mainly attributed to the protective effects of the harmone oestrogen. They also have a tendency to acquire H.Pylori infection later in their life time.

Studies carried out by Matley et al[53] states that carcinoma stomach occurring in young individuals posses certain specifications. They predominantly are of diffuse type, with signet ring morphology often affecting the female sex. They shows minimal association with intestinal metaplasia. They have increased propensity to occur in the proximal stomach.

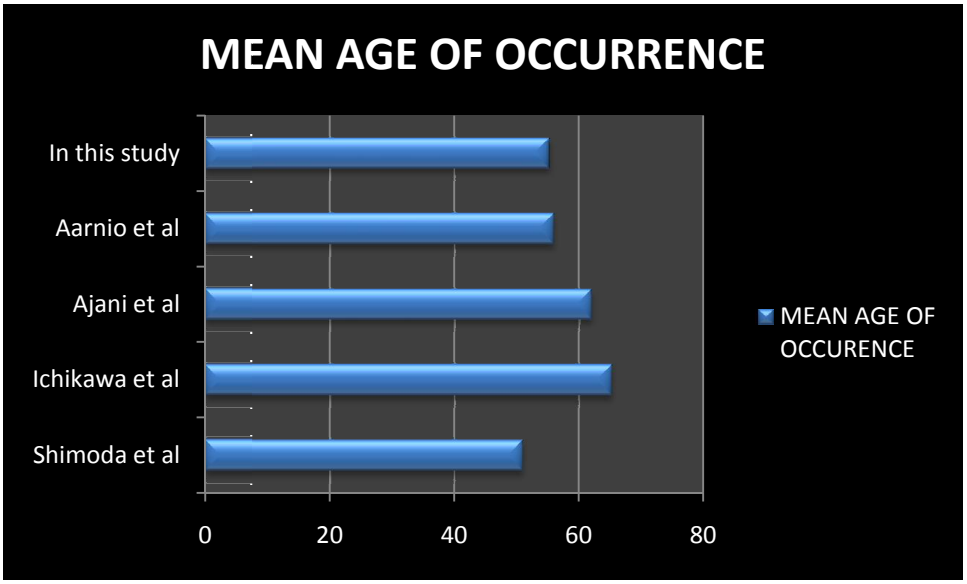
A study by Kshitiz Ranka et al[54] states that the predominant age & sex affected by stomach cancer is men in their 5th decade of life. Kalyani et al[55] studied the spectrum of gastric cancer in the Kolar district of Karnataka for a period of ten years. She concluded that the common malignancy among gastrointestinal tract was that of stomach. Maximum incidence was observed in 7th decade in males and 6th decade in females. This was in contrast to some studies which advocated late incidence in females.

TABLE 18 :COMPARISON OF MEAN AGE OF OCCURENCE OF GASTRIC CARCINOMA

STUDY	MEAN AGE OF OCCURENCE
Shimoda et al[56]	51 (34 -65 years)
Ichikawa et al[57]	65.3 (36 - 83 years)
Ajani et al[58]	62 (30 -75 years)
Aarnio et al[59]	56 (31 -85 years)
In this study	55.3 (28 -77 years)

The mean age of occurrence in this study is 55.3 years. The youngest age of occurrence was 28 years and the oldest age of occurrence in this study is 77 years.

CHART 18 : COMPARISON OF MEAN AGE OF OCCURRENCE OF GASTRIC CARCINOMA



SITE OF INCIDENCE:

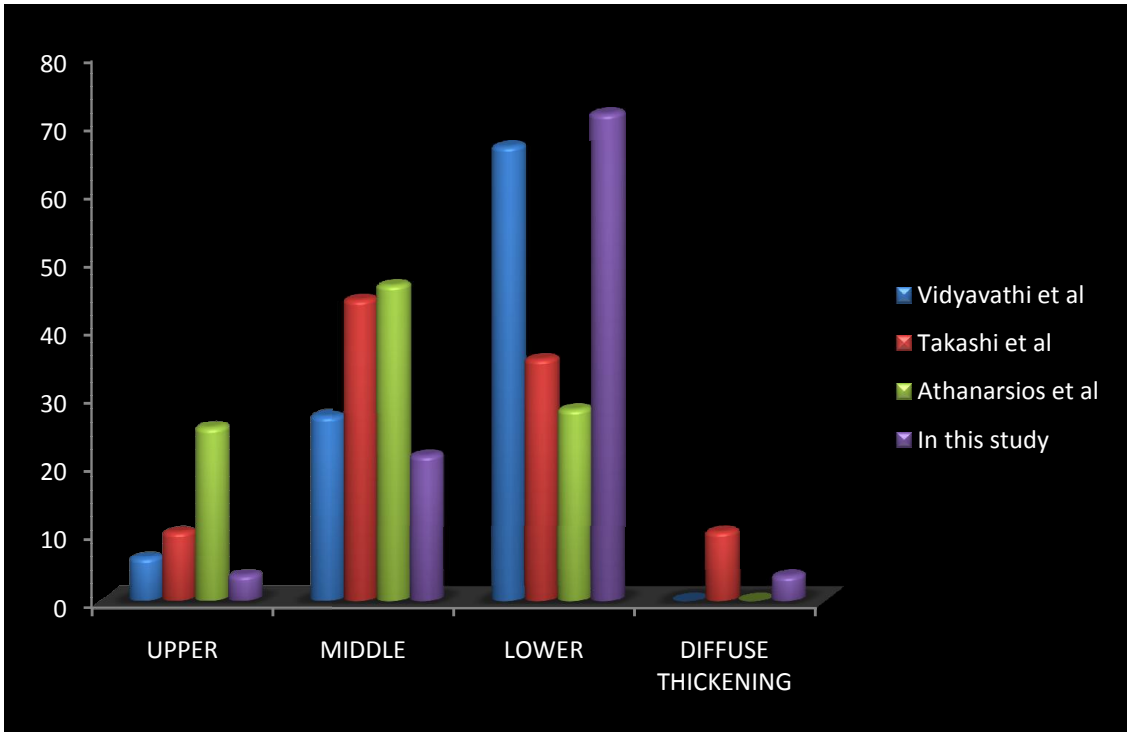
Most cases are located in the lower portions of the stomach mainly the pyloric antrum . Inoue et al[40] states that of all cases of gastric carcinoma only 15 % are situated in proximal stomach.

In another study Roder et al[38] reports an incidence of 31.7% of proximal gastric cancers in male and 18.8% of cases in females. The high risk regions of the world has a predilection to develop carcinomas in antrum and pylorus. In the low risk regions the incidence of proximal gastric cancers are found to be increasing[60].

TABLE 19: COMPARISON OF THE SITE OF OCCURRENCE OF GASTRIC CARCINOMAS IN VARIOUS STUDIES

STUDY	UPPER	MIDDLE	LOWER	DIFFUSE THICKENING
Vidyavathi et al[61]	3 (6.25%)	13 (27.08%)	32 (66.66%)	0
Takashi et al[91]	64(10.11%)	280(44.23%)	225(35.54%)	64(10.11%)
Athanarsios et al[85]	28(25.45%0	51(46.36%)	31(28.18%)	0
In this study	5(3.75%)	30 (21.43%)	100 (71.4%)	5 (3.57%)

CHART 19 : COMPARISON OF THE SITE OF OCCURRENCE OF GASTRIC CARCINOMAS IN VARIOUS STUDIES



Katherine et al[41] states that distal cancers are predominant in countries like East Asia, Central and South America. North America, Australia, New Zealand are said to have low incidence of distal tumours. Distal tumours are the predominant subtype occurring in Japan. The incidence of proximal cancers in Japan is increasing. This is attributed to the westernization of food culture.

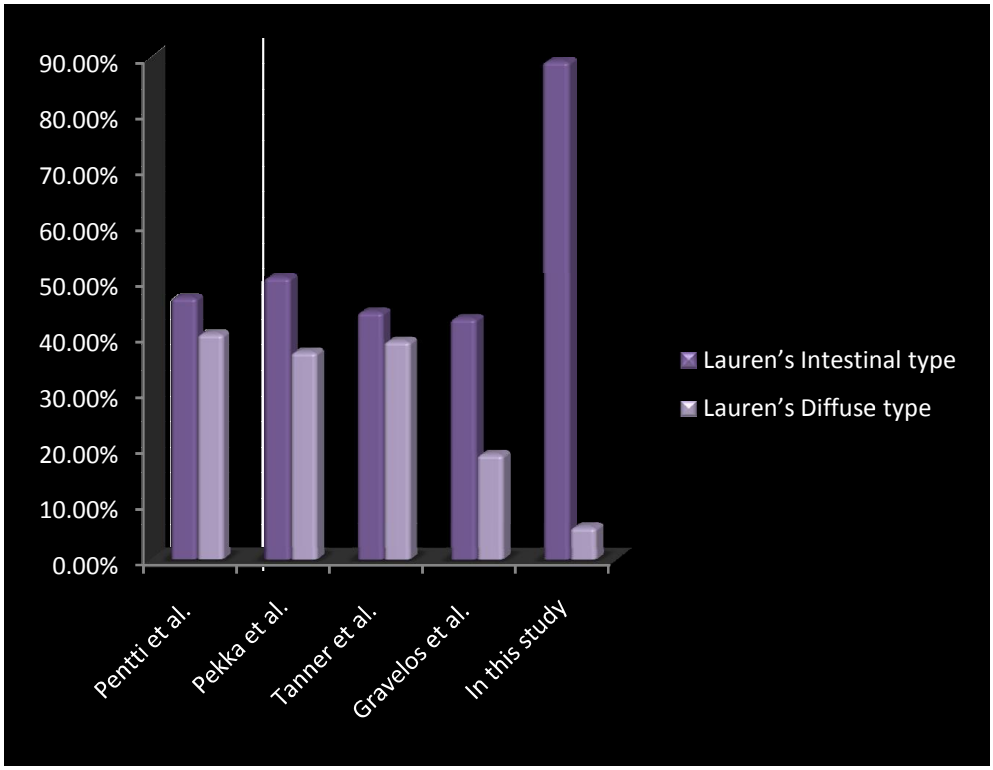
In our study the tumours of the distal portions of the stomach constituted the majority of lesions with around a 100 cases (71.4%) which correlates with most of the studies.

COMPARISON OF THE HISTOLOGICAL CLASSIFICATION

TABLE 20 :COMPARISON BASED ON LAUREN'S CLASSIFICATION

Study	Lauren's Intestinal type	Lauren's Diffuse type
Pentti et al. [62]	121/257 (47.08%)	104/257 (40.46 %)
Pekka et al.[63]	50.5 %	37.2%
Tanner et al.[31]	51/115 (44.35%)	45/115 (39.13%)
Gravelos et al.[11]	16/37(43.2%)	7/37 (18.9%)
In this study	125/140 (89.28%)	8/40 (5.71%)

CHART 20 : COMPARISON BASED ON LAUREN'S CLASSIFICATION



In this study the number of cases presenting with the Lauren's intestinal type is 125 constituting 89.28%. 8 cases come under the diffuse type of tumours constituting 5.71%.

Various classification systems have been put forth in studying gastric carcinomas. The most commonly used is Lauren's and WHO classification.

Pentti et al[62] states that in a comparative study between cases and controls intestinal metaplasia was common in malignant cases. It was around 42% when compared to non malignant counterparts (23%).

In a study of gastric cancer occurring in HNPCC syndrome patients, Aarnio et al[59] states that intestinal type gastric carcinoma had more positive co relation with chronic gastritis associated with H.pylori infection when compared to diffuse carcinomas.

A time trend study was carried out in high and low risk areas by Pekka et al[63.] It observed that during the initial time period of the study the ratio of intestinal and diffuse type of carcinoma was 1:7. This was followed by a gradual transition resulting in a ratio of 1:3 twenty years later. This transition said to be due to increase in cases of intestinal carcinoma.

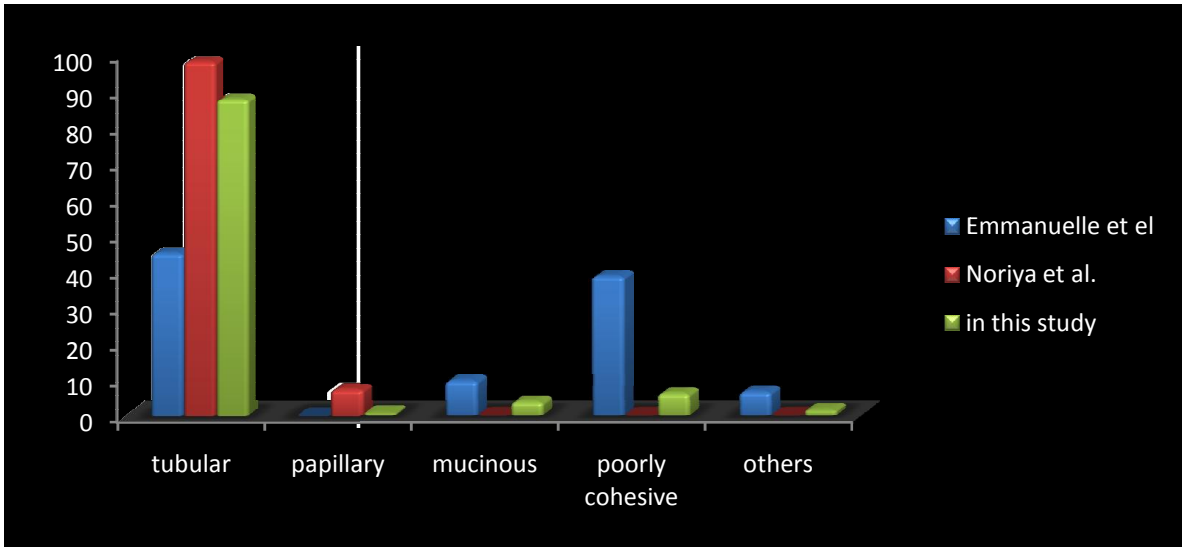
In a study analyzing the gastric carcinoma of young Tso et al[70] observed that the tumours were predominantly of diffuse type. These diffuse carcinomas cases occurred predominantly in females. Most of the patients had reduced survival rates.

In his study Si Chun Ming[71] states that the survival in patients with intestinal type carcinoma is more when compared to patients with diffuse type carcinoma.

TABLE 21 : COMPARISON BASED ON WHO CLASSIFICATION

WHO CLASSIFICATION	TUBULAR	PAPILLARY	MUCINOUS	POORLY COHESIVE	OTHERS
Emmanuelle et al[89]	14	0	3	12	2
Noriya et al[64]	129	10	0	0	0
In this study	117	1	5	8	2

CHART 21 : COMPARISON BASED ON WHO CLASSIFICATION



In this study, according to the W.H.O classification majority of the cases were of tubular adenocarcinoma constituting 117 cases (87.96%). This was followed by poorly cohesive variant of carcinoma predominantly of the signet ring cell type 8 cases (6.01%). 5 cases were mucinous adenocarcinoma (3.76%), 2 cases of adenosquamous carcinoma (1.5%) and one case of papillary adenocarcinoma(0.7%). Noriya et al[64] reported 129 cases of tubular adenocarcinoma and 10 cases of papillary adenocarcinoma.

TABLE 22 : COMPARISON BASED ON GRADE OF DIFFERENTIATION

DIFFERENTIATION	WELL	MODERATE	POOR
Nobuyuki et al[86]	9	13	9
Ferda et al[76]	7	5	4
In this study	20	69	37

CHART 22 : COMPARISON BASED ON THE GRADE OF DIFFERENTIATION

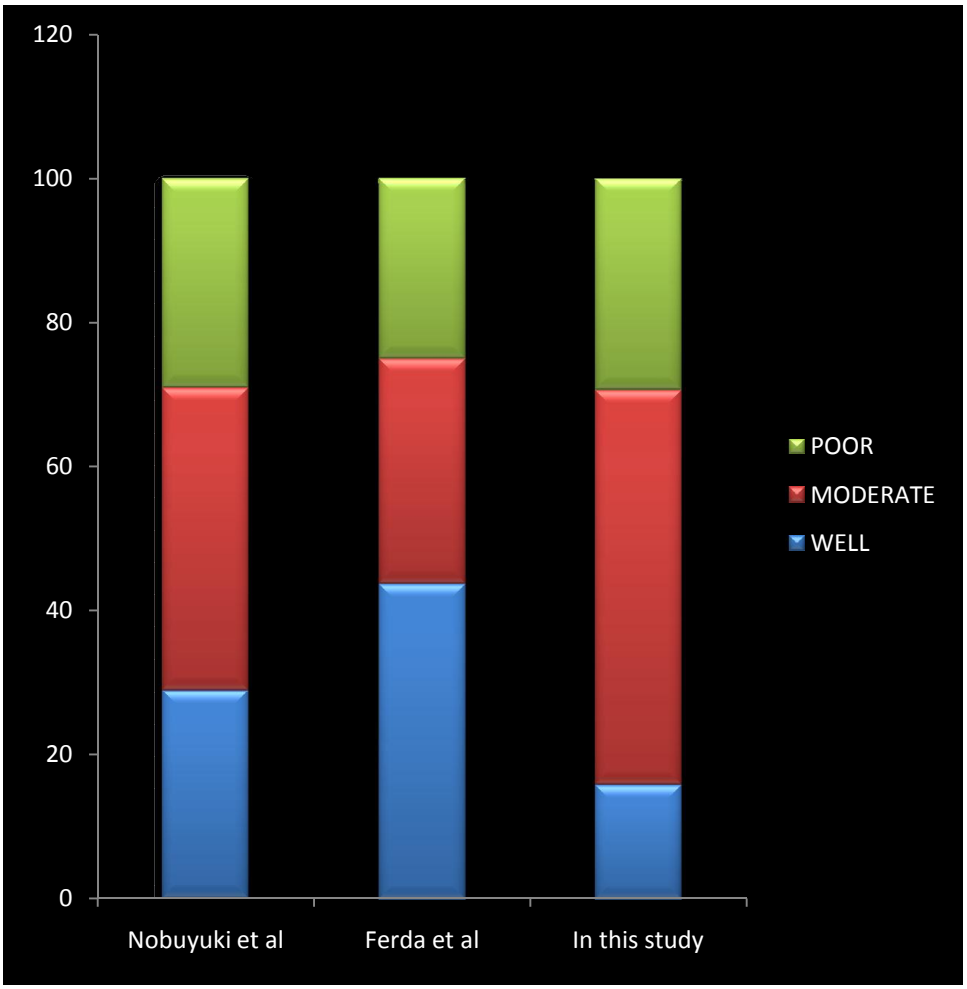
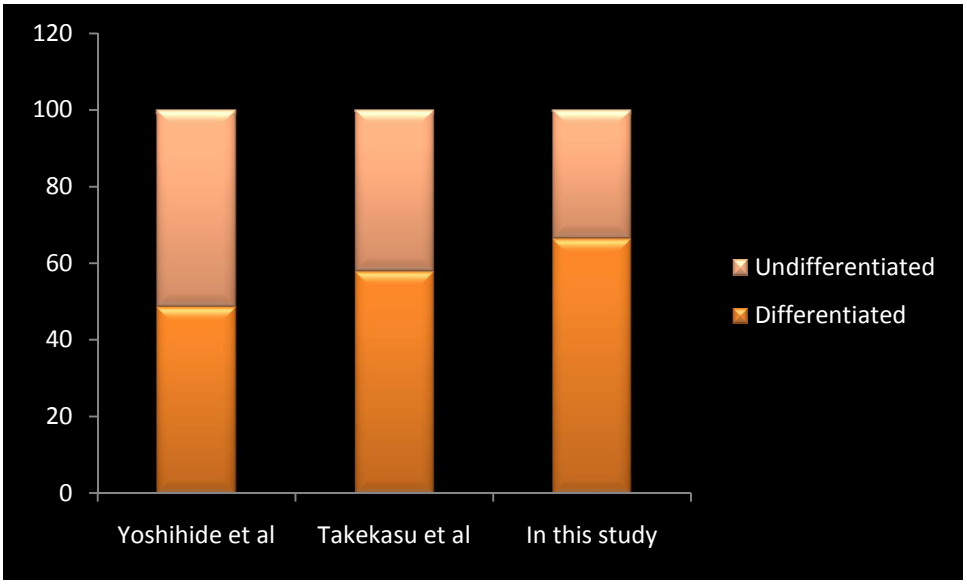


TABLE 23 : COMPARISON BASED ON NAKAMURA'S CLASSIFICATION

STUDY	DIFFERENTIATED	UNDIFFERENTIATED
Yoshihide et al.[65]	53	56
Takekazu Yamao[66]	667	486
In this study	89	45

CHART 23 : COMPARISON BASED ON NAKAMURA'S CLASSIFICATION



In this study 89 cases were classified as differentiated tumour . 45 Tumours were of the undifferentiated type. In a study carried out in the cancer institute hospital of Tokyo in early gastric carcinoma, Hirotoshi et al[67]. compares differentiated and undifferentiated carcinoma. He states that 87.8% of carcinomas studied were of the differentiated type when compared to 33.7% carcinomas which was of undifferentiated type. In a study by Kiyoshi et al[68] the penetrating type

A tumours were more differentiated (81.3%) when compared to the penetrating type B tumours which had more of poor differentiation

STAGING AND LYMPH NODE INVOLVEMENT

Takikazu et al[66] states that one of the most important prognostic factor in early gastric carcinoma is the presence or absence of lymph node involvement. He states that the occurrence of nodal metastasis in early gastric carcinoma is rare.

Noriya et al[64] studied the clinical outcome of patients who underwent endoscopic mucosal resection for early gastric carcinoma. It was observed that the lymphatic involvement was seen in 1 case of carcinoma confined to mucosa and 13 cases with extension of carcinoma into the sub mucosa. The most significant determinant for lymph node metastasis in early gastric carcinoma was venous or lymphatic invasion. Increased lymph node metastasis was observed in case of papillary adenocarcinoma.

Yoshihide et al[65] in his study evaluated the E Cadherin expression in gastric carcinoma. He observed that cases with increased nodal metastasis and distant organ involvement had increased expression of E Cadherin.

Jurgen et al[69] in a German gastric carcinoma study observed that the prognosis was bad in gastric carcinoma if 20% of the resected nodes showed evidence of metastasis. He stated that the extent of lymph node dissection could be considered one of the independent prognostic factor in gastric carcinoma in addition to lymph node status, depth of invasion and residual disease.

TABLE 24 : EXTENT OF TUMOUR INVASION

TUMOUR INVASION	TX	T1	T2	T3	T4
Jian Chen et al[87]	7(10%)	8(12%)	2(3%)	50(74%)	1(1%)
Young En Yoo et al[84]	0	16	29	61	13
In This study	0	10	18	8	14

CHART 24 : EXTENT OF TUMOUR INVASION

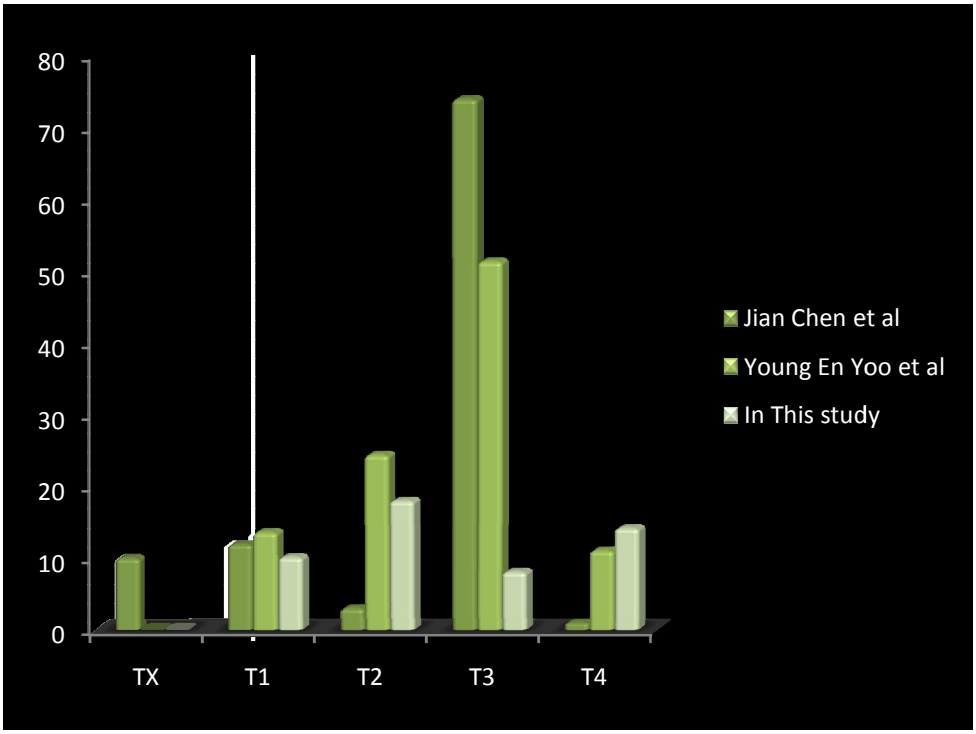
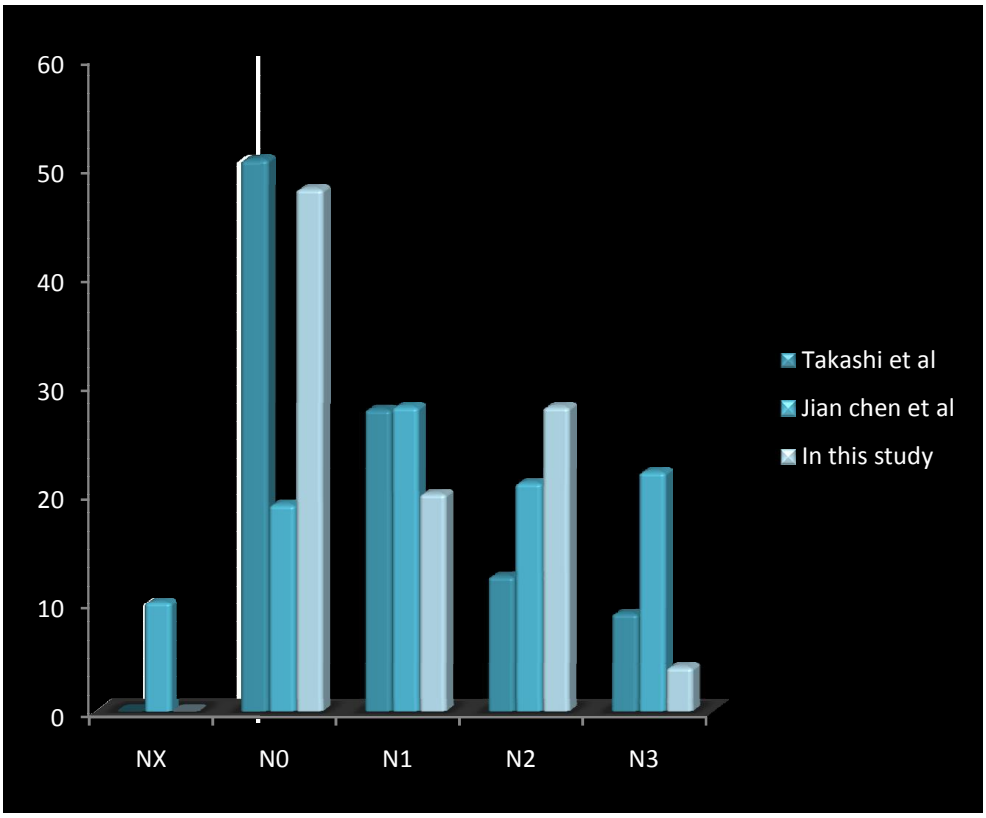


TABLE 25 :COMPARISON OF LYMPH NODE STATUS BASED ON TNM CLASSIFICATION

NODAL STATUS	NX	N0	N1	N2	N3
Takashi et al[91]	0	298(50.76%)	163(27.76%)	73(12.43%)	53(9.02%)
Jian chen et al[87]	7(10%)	13(19%)	19(28%)	14(21%)	15(22%)
In this study	0	24(48%)	10(20%)	14(28%)	2(4%)

CHART 25 : COMPARISON OF LYMPH NODE STATUS BASED ON TNM CLASSIFICATION



HER 2 Neu IN GASTRIC CARCINOMA

10% of the world's malignant tumour burden is contributed by gastric carcinoma. The vague and nonspecific symptoms presents majority of cases in an advanced stage. These tumours are often unresectable. In the modern treatment era, cancer is one disease where treatment regimens are tailored to suit the need of the individual patient. This includes an array of combination chemotherapy coupled with targeting the molecular basis of the disease pathogenesis.

Several molecular agents have been tested for the targeted therapy . They are Cetuximab, Matizumab, Panituzumab, Trastuzumab etc. They act via the Epidermal Growth Factor receptor. Gefitinib, Erlotinib and Lapatinib act as tyrosine kinase inhibitors. Other agents that can be used are matrix metalloprotease, Insulin like growth factor 1, fibroblast growth factor etc.

The first drug that was used as a targeted therapy in gastric carcinoma was Trastuzumab. It got the approval of FDA in 2010. Evaluation of expression of HER 2 in tissues is an important task. This view has also been supported by the ToGA trials.

The family of Epidermal Growth Factor Receptors is formed by four members. They undergo activation by ligand binding. This results in homo or heterodimerization. Of all these receptors HER 2 is unique.It has a special conformation that makes it constitutively active. When over expressed it serves as the most likely binding partner to other receptors of same family. This initiates signal transduction that triggers uncontrolled cell proliferation. HER 2 over expression in Gastric carcinoma is said to have a positive correlation with shorter survival. In the ToGA trail the percentage of membrane over expression was 22.1%

Specificities of HER 2 over expression in gastric carcinoma

1. There is heterogenous distribution of HER 2 over expressing cells in gastric carcinoma.
2. The expression of HER2 in gastric carcinoma is less when compared with tumours of the oesophagogastric junction.
3. HER2 over expression is more in intestinal type of carcinoma when compared to the diffuse type.

According to the reports of Zhang et al HER3 over expression of 26.6% has been observed in diffuse carcinomas and dedifferentiated carcinomas.

Importance of determining HER 2 positivity

The aim of determining the HER 2 status is not only to effectively implement the drug therapy that is costlier than the most conventional regimens but to effectively exclude the HER 2 negative individuals because of the accompanying side effects of trastuzumab.

HER2 over expression with immunohistochemistry should be positively co related with FISH, CISH, RT- PCR. The extracellular domain of HER2 can be measured in the serum samples using ELISA technique.

Factors influencing HER 2 expression in gastric carcinoma

1. Fixative used
2. Fixation time
3. Thickness of the tissue slide
4. Time interval between preparation and staining of sections
5. Method used for antigen retrieval.
6. Type of antibody used.
7. Dilution of the antibody employed

8. Temperature for incubation
9. Time for incubation
10. Scoring system employed.

Alberello L et[72] stated that the degree of correlation between HER 2 amplification and expression is less in gastric carcinomas. Around 20% of cases show amplification but no expression of the protein. These patients may not benefit from Trastuzumab. Hence IHC should always be the first line of investigation. The role of the pathologist is crucial in selecting the therapy. They providing accurate and reproducible results.

Grabsch et al[73] documented that 10% of the tumours showed a positive HER 2 expression. Of these tumours 91% were of intestinal type. No appreciable co relation was observed between HER 2 status, staging and survival of patient. The study also reported prominent intratumoral heterogenicity. These type of lesions have a propensity to produce false negative results when HER2 testing is done on endoscopic biopsies.

BangYJ et al[74] observed the mean standard survival rate was 18.6 months in a group of patients who received trastuzumab. This is compared to 17.1 months in a group who received chemotherapy alone.

Federica et al[75] observed no significant correlation in the degree of over expression of HER2 with respect to the site, differentiation, pattern of growth and staging .According to this study IHC 0 to 1+ had a negative predictive value of 80 and a positive predictive value of 78.6. Of the 54 cases 49 cases were completely negative and out of the positive cases only 13 cases (24%) had homogenous expression.

Jan Trost Jorgensen et al[10] in his study analysed 42 publications regarding expression of HER2 in gastric carcinoma. 71% of the studies implied a positive

corelation between HER2 overexpression and parameters like nodal involvement, serosal extension and distant metastasis.

Gravalos et al[11] observed that the expression of HER2 differed depending on histological type of tumour. HER2 expression was 16% for intestinal adenocarcinoma, 7% for diffuse type adenocarcinoma and not known for 14%. The HER 2 expression was 9.5% expression in tumours of stomach when compared to 25% expression of cancer in gastrooesophageal junction.

Tanner et al[31] studied the HER 2 amplification in gastric carcinoma using Chromogenic in situ Hybridisation. It was documented that HER 2 expression was present in 16 (12.2%) of the total 131 cases of gastric carcinomas. The HER2 amplification is more with intestinal type than the diffuse type. (21.5% vs 2%)

Josef Ruschoff et al[13] reported HER2 positivity rate of 21% in gastric carcinoma when compared with tumours of gastro oesophageal junction. Co relation with fluorescent insitu hybridization in all 3+ cases showed 100% amplification when compared to 32% of 2+ cases and 5% of 1+ cases. 497 cases were analyzed and 22.8% of the specimens showed HER 2 positivity.

Ferda et al[76] observed 3+ HER2 positivity that accounted for 24% of the cases studied. The expression co related with the intestinal type of carcinomas. The HER2 expression was also increased in tumours in an advanced stage of 3 & 4.

Fusco et al[77] observed that out of 292 cases studied 51 cases had a score of 2+ and 3+. 27 cases had a score of 3+(8%). 29 cases had a score of 1+ and 212 cases had a score of 0 when their HER2 expression was analyzed comparing with nodal metastasis. 14% of cases were discordant with initial results. 22 cases that had a positive HER2 score in primary gastric cancer had a negative score in the nodes. Among the 126 cases of primary tumours that were HER2 negative primary tumour 6 cases showed HER 2 positive lymph nodes.

Celick et al[78] in his study observed that 11 out of 62 cases showed strong membrane positivity of 17.7% the increased expression was more in intestinal type carcinomas (24.3%) when compared to diffuse carcinomas exhibiting 4.76%. There was no co relation with the histological type, extent of invasion, size of tumour, nodal metastasis and HER2 expression. There was no relation between the proliferative activity and c-erb-2 expression. Hence it was concluded that c-erb-2 cant be used as a diagnostic marker in gastric carcinoma.

TABLE 26 : COMPARISON OF HER 2 POSITIVITY IN VARIOUS STUDIES

STUDY	PERCENTAGE OF POSITIVE CASES
Ayre Dursin et al[78]	17.7%
Gravalos et al[11]	9.5%
In this study	8%

Josef Ruschoff et al[13] in his study observed that out of 8 cases only 2 cases were HER2 positive. 6cases were reported to be equivocal. In this study out of 25 cases 2 cases had HER 2 positivity with a IHC score of 3+. 2 cases were equivocal with a scoring 2+. 21 cases had a score of 0 or 1+ and were considered to be negative.

CHART 26 : COMPARISON OF HER 2 POSITIVITY IN VARIOUS STUDIES

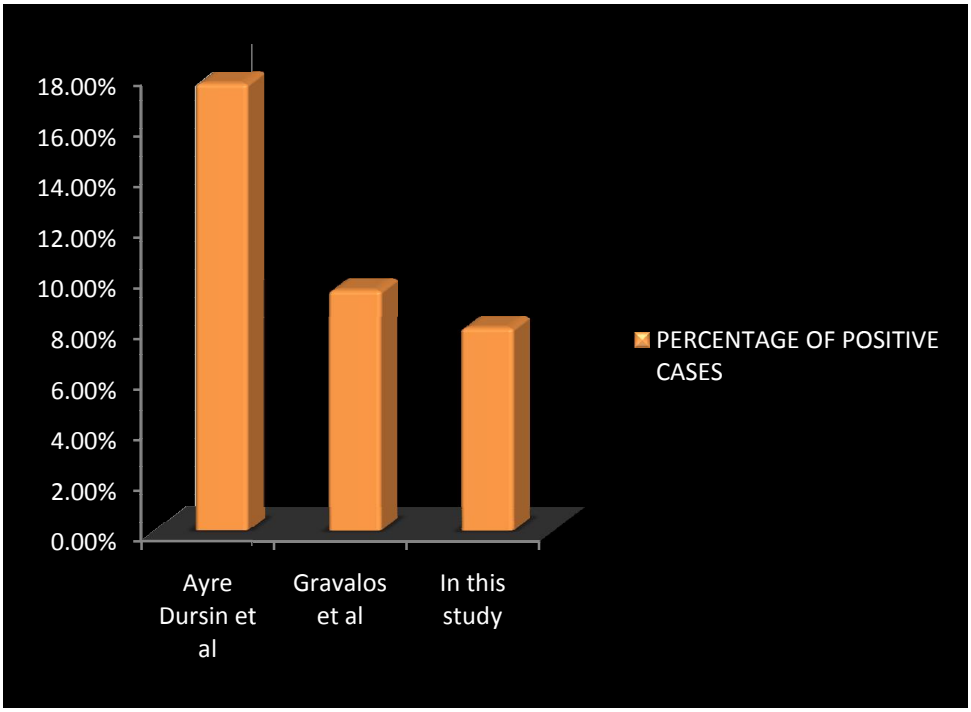
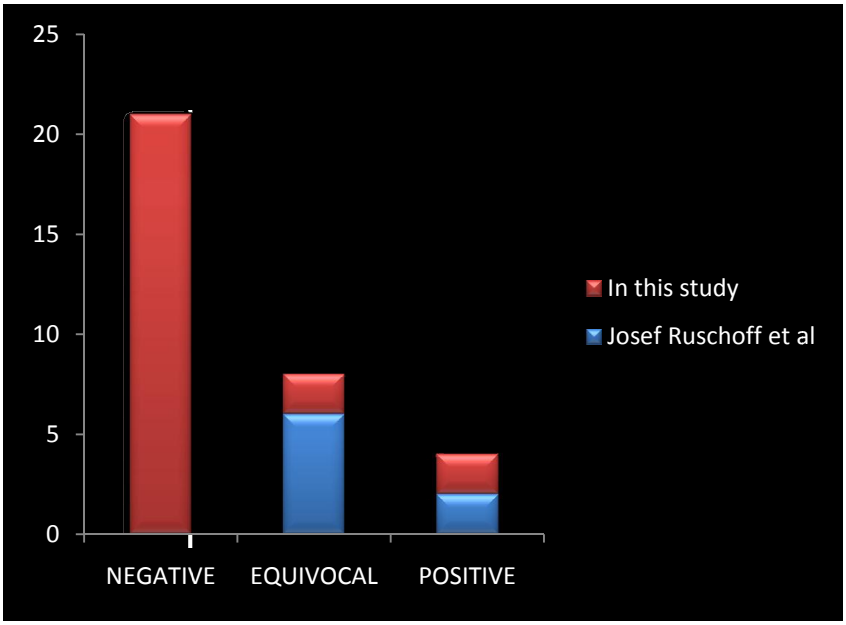


TABLE 27 : COMPARISON OF HER2 SCORE WITH OTHER STUDIES

STUDY	NEGATIVE	EQUIVOCAL	POSITIVE
Josef Ruschoff et al[13]	0	6	2
In this study	21	2	2

CHART 27 : COMPARISON OF HER2 SCORE WITH OTHER STUDIES



ROLE OF Ki 67 IN GASTRIC CARCINOMA

The proliferative activity of a tumour can be determined by various methods namely

1. Cell cycle phase distribution using flow cytometric analysis.
2. Cells in S – phase fraction using thymidine labeling index.
3. Bromo deoxy uridine labeling index.
4. Estimation of the mitotic activity.

Each techniques comes with its own disadvantage. Mostly the amount of material obtained would be small. The process of administering a mitogenic agent like bromodeoxyuridine and iododeoxyuridine is a time consuming and complex. The technique of flow cytometry to determine the proliferative activity may not be precise. This technique not only measures the proliferative activity of tumour cells. It takes into account the proliferative activity of the stromal cells, inflammatory cells such as the plasma cells and lymphocytes.

Yutaka yonemura et al[80] states that Ki 67 labelling is quicker ,simple and more sensitive. It is compared to other techniques such as labeling with H. Thymidine or Bromodeoxyuridine. Ki 67 labelling does not require incubation of a fresh tissue sample.

Ki 67 is a monoclonal antibody that reacts with the nuclear antigen. It is expressed throughout the the cell cycle, mainly in the late phase of G_1, S, G_2, and M. It is absent in the resting cells of the G_0 phase. It is said to resemble the monoclonal antibodies of DNA polymerase. The Ki 67 positive cells are situated in and around the mucous neck zone in the normal gastric mucosa.

Yoshihiro et al[81] documented that tumours in advanced stage of pT3 and pT4 had elevated Ki 67 labelling index when compared to the tumours in stage pT1 and pT2.

Ki 67 expression was higher in tumours (>30.4%) with nodal metastasis and lymphovascular invasion. Tumours with increased metastatic potential had increased Ki 67

expression when compared to tumours not going for a distant metastasis. Tumours in stage pT1 and pT2 had a Ki 67 labeling index of <30.4% in 24 cases and >30.4% in 16 cases. Tumours with stage pT3 and pT4 had 19 tumours with Ki 67 activity of <30.4% and 32 tumours with a Ki 67 activity of more than >30.4%. 43 cases that had a proliferation index of >30.4% showed lymph node metastasis when compared to five cases with >30.4% labeling index having negative nodal involvement. Similarly vascular invasion was positive in 40 cases with a Ki 67 labelling of >30% when compared to 8 cases that had no vascular invasion.[81]

Rosa et al[82] observed that the proliferative index of a gastric malignancies co related with both Ki 67 and Proliferating Cell Nuclear Antigen. PCNA is a 36 KD nuclear protein that is closely associated with the cell cycle. This gets expressed in the nuclei of cells in all phases of the cell cycle. It is produced in the late G1 and S phases. PC 10 is the monoclonal antibody that is produced against the genetically engineered PCNA. PCNA is an auxiliary protein of DNA polymerase δ. This was accidentally discovered when the auto antibodies in SLE reacted with them.

According to Rosa et al[82] no significant co relation was obtained between PCNA expression and Ki 67 labeling. This could be attributed to the marked intertumoral heterogenicity, sample size ,tissue preparation techniques etc. The ability of PCNA to identify cells that has left the cell cycle recently because of its long half life and dysregulated expression of the DNA in malignancy.

In his study Yonemura et al[80] documented that high Ki 67 labeling index was found in 65% of cases that had lymph node metastasis and in 33% of cases that did not have a nodal metastasis. 56% of advanced gastric tumours showed high Ki 67 labeling index when compared to 37% of cases with early gatric carcinoma. Well differentiated tumours showed 47% positivity when compared to 50% of poorly differentiated tumours. 72% of cases with increased Ki 67

labeling index showed vascular invasion and 57% of cases showed positive lymphatic invasion. But no co relation was obtained between the degree of Ki 67 labeling and the gross morphology.

Chen et al[83] used a 10% cut off point for Ki 67 nuclear positivity to differentiate the patients with over expression and under expression. In his study over expression of Ki 67 was found in 74.42% of cases with survival of 43.31% for a period of three year and 35.94% survival for a period of 5 years. This was lower in individuals with a negative or a low Ki 67 expression which was 77.27% for 5 years and 72.73% for 5 years. He states that the degree of over expression of Ki 67 in gastric carcinoma has Co relation with the grade, extent of invasion and clinical stage. It was found that tumours of stage III and IV showed higher expression of Ki 67 proliferation index when compared to tumours in stage I and II. According to Chen et al[83] Ki 67 is a useful indicator in predicting the proliferative capacity of the tumour cells and also the extent of differentiation.

Young Eun Joo et al[84] in his study of 119 cases observed that the percentage of expression of Ki 67 ranged from 7.6% to 85.8%. The cut off point that was used to categorize the cases into high and low Ki 67 expression was obt ained by calculating the mean and the mean valve of 49.5 was obtained. Accordingly 57 pateints were categorized in the group showing high expression and 62% of patients had low Ki 67 expression

Athanauio S et al[85] in his study observed that increased Ki 67 labelling was observed in high grade tumours and tumours that had reached an advanced stage at the time of clinical presentation. According to his study tumours that had a Ki 67 expression of<5% and negative expression for Bcl 2 had higher survival rates when compared to Bcl 2 negative tumours with Ki 67 expression of >5%. Ki 67 over expression is associated with poor survival and is considered to be one of the independent prognostic factors in his study.

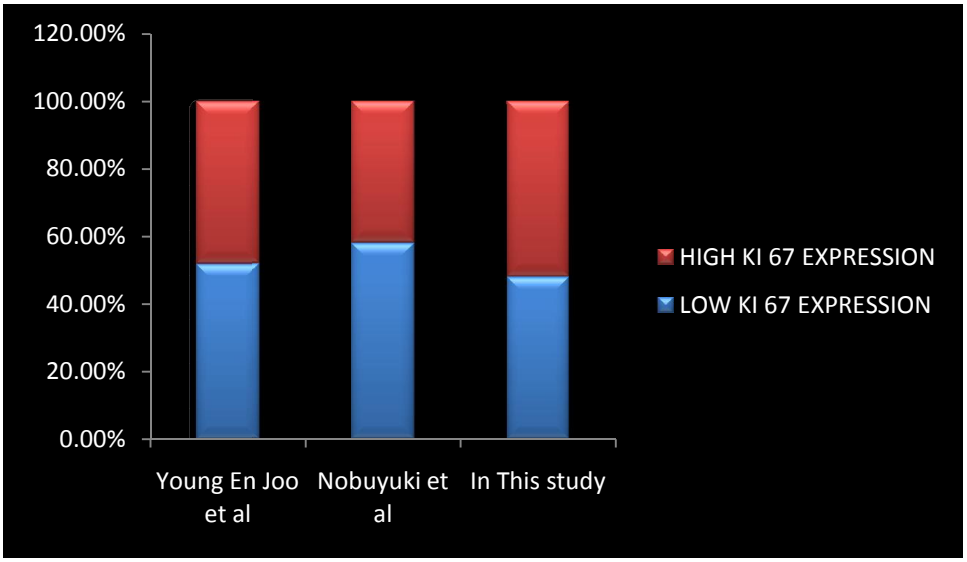
Dursun et al[78] studied the expression of c-erb and Ki 67 in patients with gastric carcinomas. He documented that no significant correlation was obtained between the degree of c-erb expression and the proliferative activity documented by Ki 67 labeling.

Nobuyuki Igarashi et al[86] stated that patients with advanced gastric cancer had higher Ki 67 labeling index when compared to the mean value of 38.3%.

TABLE 28 :COMPARISON OF KI 67 EXPRESSION IN VARIOUS STUDIES

STUDY	LOW KI 67 EXPRESSION	HIGH KI 67 EXPRESSION
Young En Joo et al[84]	62 (52.10%)	57(47.89%)
Nobuyuki et al[86]	18(58.06%)	13(41.93%)
In This study	12(48%)	13(52%)

CHART 28 : COMPARISON OF KI 67 EXPRESSION IN VARIOUS STUDIES



SUMMARY

SUMMARY

This study was done in the Department of Pathology, Thanjavur Medical College from the period of July 2012 to June 2014. 260 samples were received including both endoscopic biopsies and gastrectomy specimens. Using the exclusion criteria only 140 samples were included in this study. The results of this study is summarized as follows:

- The incidence rate of gastric carcinomas in this study is 6.78/1000 cases.
- The most commonly affected age group is 50 -60 years constituting 34.28%
- Males are more commonly affected than females with the male female ratio of 2.3:1.
- Out of 127 endoscopic biopsies in male frank malignancy was reported in 45.6% of cases.
- Out of 45 endoscopic biopsies in female frank malignancy was reported in 40% of cases.
- The most common site of malignancy in this study is the gastric antrum. Around 60% of malignant lesions were localized here.
- The least common site of gastric malignancy is the cardia constituting only 3.57% of cases in that location.
- The most common subtype of malignancy according to the WHO classification is the Tubular adenocarcinoma. (83.57%)
- The most commonly observed microscopic subtype, according to Lauren's classification is the intestinal type adenocarcinoma (89.28%)
- Most of the tumours have extended to the muscularis layer at the time of surgical resection (36%)
- Majority of the cases showed no nodal involvement (48%)

- Out of 25 case studied for Immunohistochemistry HER 2 positivity was seen in 2 cases (8%).HER2 scoring of 3+ was seen predominantly in well differentiated type of intestinal adenocarcinoma.
- Out of 25 cases analyzed for Ki 67 expression, 52% of cases had low Ki 67 index . High Ki 67 expression was seen in 48% of cases.
- There was no significant co relation observed between the grade of differentiation and the proliferation index.

LIMITATIONS OF THIS STUDY

- Open to selection bias.
- Small sample size.
- Limited information regarding clinical details and follow up.
- Potential for deterioration of antigenicity in the samples for IHC due to long period of storage.
- Inter observer variation in interpretation of IHC results.

CONCLUSION

CONCLUSION

Gastric carcinoma is one of the most devastating malignancies of the world. Its incidence has been reported from time immemorial. It still continues its reign in being one of the major killer diseases of mankind. The morbidity and mortality arising from this particular tumour is vast. This study done in our institution is a step towards understanding the behavior of this disease in the current scenario.

Gastric carcinoma is one of the malignanies to present in an advanced stage in a majority of cases. This is mainly attributed to the non specific symptoms accompanying it. This delays the timely diagnosis, which further delays the treatment.

Thanjavur Medical college, caters to the health care needs of a mixed population of urban and semi urban people.This study documents the details regarding the incidence, age distribution, sex ratio, percentage of malignant lesions, predominant site, histological type , Tumour grade, extent of serosal invasion and nodal metastasis of the cases of gastric carcinoma diagnosed and treated here.

In this modern era, therapies are tailor made to suit the needs of the individual patient. Concepts of targeting the molecular basis of the disease in gaining popularity. In this study two such markers were chosen. . Ki 67 is a proliferation marker. Its percentage of expression throws light in the aggressiveness of the tumour. HER2neu is a marker which is gaining importance in gastric cancer. Documentation of its level of expression in gastric carcinoma helps in using Trastuzumab, a monoclonal antibody against the HER2 receptor. This could be useful in patients who often presents in an advanced inoperable stage of the disease.

ANNEXURES

APPENDIX I

HEMATOXYLIN AND EOSIN STAIN

Preparation of solution :

HARRIS HEMATOXYLIN

- Distilled water :1000ml
- Ammonium alum : 100g
- Absolute ethyl alcohol : 50 ml
- Mercuric oxide :2.5g
- 100g of ammonium alum is dissolved in 1000 ml of distilled water by heating at 60^0 C. add solution of 5g of hematoxylin in 50 ml of ethyl alcohol and bring rapidly to boil. When it begins to boil remove from flame and add 2.5 g of mercuric oxide. Mix by swirling gently.

EOSIN STAIN

- Eosin Y : 1 g.
- Distilled water : 20 ml
- 95% ethanol : 80 ml
- Glacial acetic acid : 0.2 ml
- Dissolve 1g of eosin Y in 20 ml of water, then add 80 ml of 95% ethyl alcohol and 0.2 ml of glacial acetic acid.

Procedure :

1. Bring the sections to water
2. Dip in Harris hematoxylin for 15 minutes.
3. Rinse in tap water.
4. Differentiate in 1% acid alcohol- 3-4 quick dips.
5. Wash in tap water briefly.
6. Dip in ammonia water or saturated lithium carbonate until the sections are blue.
7. Wash in running tap water for 10 -20 minutes.
8. Stain with eosin for 15 seconds to 2 minutes depending upon the age of eosin and the depth of counter stain.
9. Rinse in tap water.
10. Dip in 95% alcohol.
11. 3 changes in absolute alcohol.
12. Xylene – 2 changes.
13. Mount in DPX mountant.

APPENDIX II

PERIODIC ACID SCHIFF TECHNIQUE

Solutions required

a) 0.5% periodic acid

b) Mayer's haemalum

c) Sulphorous acid

Sodium metabisulphite 10% 6 ml

N/10 Hydrochroric acid 10% 5 ml

Distilled water 100 ml

d) Schiffs Reagent

Basic Fuschin 1 gm

Sodium metabisulphite, anhydrous 1 gm

Distilled water 200 ml

N/10 Hydrochloric acid 20 ml

Boil the distilled water, add the basic fuschin. Stir and cool to 50^0 C. Filter and add hydrochloric acid, cool to 25^0 C and add Sodium metabisulphite. The solution becomes ready for use when it becomes nearly colourless. This process may take upto two days in the dark. (Alternatively activated charcoal may be added to the solution, shaken and filtered). When the solution becomes discoloured it should be discarded.

Technique

1) Bring the sections to water.
2) Periodic acid 0.5% for 5 minutes
3) Rinse in distilled water
4) Schiff's reagent 15 minutes

5) Rinse in three fresh changes of Sulphorous acid 2 minutes in each change for 6 minutes.
6) Wash in running tap water each change 5 minutes
7) Counter stain in Mayer's haemalum 30 seconds
8) Wash in running tap water 5 minutes
9) Dehydrate, clear and mount

Results

Neutral mucin – Magenta, Nucleus – blue.

APPENDIX III

IMMUNOHISTOCHEMISTRY

PREPARATION OF SOLUTIONS:

CITRATE BUFFER SOLUTION- ANTIGEN RETRIVAL SOLUTION

Trisodium citrate : 2.94 gms

1 N Hydrochloric acid : 5 ml

Required pH is 6.0, that is obtained by titration with 1N HCl.

TRIS BUFFER SALINE (TBS) – WASH BUFFER

Sodium chloride : 8 gms

Tris (hydroxymethylamine) : 0.605 gms

1 N Hydrochloric acid : 4 ml

Distilled water : 1 litre

Required pH is 7.6, that is obtained by titration with 1N HCl.

PREPARATION OF CHROME ALUM COATED SLIDES

Potassium dichromate : 50 mgs

Gelatin : 300 mgs.

Distilled water : 100 ml.

Potassium dichromate is added to distilled water and then boiled to 60^0 C. gelatin is then added slowly to it. Glass slides are then dipped in this solution and dried overnight.

After taking the required sections into the coated slides, it is baked overnight at 45^0 C in the autoclave. The slides are used for the procedure the next day.

ANTIGEN RETRIVAL:

The slides are arranged in a metal rack and placed in citrate buffer in a pressure cooker and allowed to boil up to three whistles.

PROCEDURE :

- Dewax the sections in xylene (15 minutes each, 2 changes) and then in decreasing grades of alcohol then finally bring the sections to running tap water followed by distilled water.
- Antigen retrival using TBS by pressure cooker method.
- Cool to room temperature in running tap water for 20 minutes.
- Wash in TBS – 2 changes for five minutes each.
- Drain and cover the tissues sections with power block for 15 minutes.
- Drain and blot the excess power block.
- Cover the sections with primary antibody for 90 minutes.
- Wash in TBS – 2 changes for 5 minutes each.
- Drain and cover the tissue sections with secondary antibody (HRP- Horse raddish peroxidase) for 30 minutes
- Wash in TBS -2 changes for 5 minutes each.
- Drain and cover the tissue sections with DAB (Diamino Benzidine) substrate buffer for 5- 10 minutes.(depending on the time suggested in the supplied kit.)
- Wash in distilled water, counter stained with hematoxylin, clear in xylene and mount with DPX.

APPENDIX –IV

TNM STAGING SCHEME FOR GASTRIC CARCINOMA

PRIMARY TUMOUR (T)	
TX	Primary tumour cannot be assesed
T0	No evidence of primary tumour
Tis	Carcinoma in situ: intra epithelial tumour without invasion into lamina propria
T1	Tumour invades lamina propria, muscularis mucosae or submucosa
T1a	Tumour invades lamina propria or muscularis mucosae
T1b	Tumour invades submucosa
T2	Tumour invades muscularis propria
T3	Tumour penetrates sub serosal connective tissue without invasion of visceral peritoneum or adjacent structures
T4	Tumour invades serosa(visceral peritoneum) or adjacent structures
T4a	Tumour invades serosa (visceral peritoneum)
T4b	Tumour invades adjacent structures
REGIONAL LYMPH NODES (N)	
NX	Regional lymphnodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1-2 regional lymph nodes
N2	Metastasis in 3-6 regional lymph nodes
N3	Metastasis in 7 or more regional lymph nodes
N3a	Metastasis in 7-15 lymph nodes
N3b	Metastasis in 16 or more regional lymph nodes
DISTANT METASTASIS (M)	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

STAGE GROUPING FOR GASTRIC CARCINOMA

Stage 0	**Tis**	**N0**	**M0**
Stage IA	**T1**	**N0**	**M0**
Stage IB	**T2**	**N0**	**M0**
	T1	**N1**	**M0**
Stage IIA	**T3**	**N0**	**M0**
	T2	**N1**	**M0**
	T1	**N2**	**M0**
Stage IIB	**T4a**	**N0**	**M0**
	T3	**N1**	**M0**
	T2	**N2**	**M0**
	T1	**N3**	**M0**
Stage IIIA	**T4a**	**N1**	**M0**
	T3	**N2**	**M0**
	T2	**N3**	**M0**
Stage IIIB	**T4b**	**N0**	**M0**
	T4b	**N1**	**M0**
	T4a	**N2**	**M0**
	T3	**N3**	**M0**
Stage IIIC	**T4b**	**N2**	**M0**
	T4b	**N3**	**M0**
	T4a	**N3**	**M0**
Stage IV	**Any T**	**Any N**	**M1**

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